Tomas Bata University in Zlín
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THE EFFECT OF COOKING ON NUTRITIVE QUALITY OF SELECTED LEGUMES

DOCTORAL THESIS

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ABSTRACT- ENGLISH

The scope of this dissertation is primarily to find the ways to make the best use of legumes to alleviate the problem of protein energy malnutrition and full fill the nutritional needs of the global population.

Properties of legumes namely, *Pisum sativum* (Terno, Xantos, Svit, Achat), *Glycine max*, *Lupinus albus* (Amiga), *Pisum sativum* var. *arvense* (Arkta), *Faba vulgaris* (Piestansky) were studied with the objectives to characterisation of legumes, to investigate the protein quality of cotyledons and radicles (shoot) after germinating for 48 hours and to study the nutritional quality of cooked legumes by normal, pressure and microwave cooking. Cooked samples were analysed in four cooking times, different in each of the methods of cooking, with the aim to increase protein digestibility. Methods used for the investigation were basic chemical composition; analysis of amino acids with ion exchange chromatography with post column ninhydrin-based detection, protein fractionation, *in vitro* protein digestibility and dry matter digestibility.

According to basic chemical composition of all legumes, it was revealed that these legumes are a rich source of protein (21.5-34.4%) with higher level of albumins including all other nutrients. The ranges of the albumin, globulin, prolamin, glutelin and residue were 40.3-48.5%, 38.6-42.0%, 3.5-5.3%, 3.4-6.4% and 2.8-7.2% respectively of the total protein. The highest amino acid content in cotyledons and radicles were noted in *P. sativum* (Xantos).

In vitro dry matter and protein digestibility of raw seeds ranged from 51.1-71.5% and 54.1-75.0% respectively. *In vitro* protein digestibility of cotyledons and radicles was above 80% and above 86% respectively. Therefore, germination of legumes for 48 h could be used as a simple method to improve the quality of protein of legumes.

Pressure cooking and microwave cooking within 8-14minutes could be recommended after soaking 0.2% $NaHCO_3$ to reduce the cooking time as well as to maintain high quality of protein for legumes. Pressure cooking (8-12 minutes) could be used as the most effective way to utilize and to improve the quality of protein of legumes. Based on the nutritive improvements after germination and cooking, it was revealed that studied *P. sativum* could be used as an alternative to *G. max*.

ABSTRACT- CZECH

Cílem dizertační práce bylo nalezení způsobu, jak zlepšit využívání luštěnin konzumenty, a tím přispět k řešení globálního problému hladu a podvýživy. Luštěniny jsou potenciálním zdrojem bílkovin právě pro rozvojový svět a mohou snížit projevy tohoto globálního problému.

Byly studovány vlastnosti 8 luštěnin: *Pisum sativum* (odrůdy Terno, Xantos, Svit, Achat), *Glycine max*, *Lupinus albus* (odrůda Amiga), *Pisum sativum* var. *arvense* (odrůda Arkta) a *Faba vulgaris* (odrůda Pieštiansky). Do studie byly rovněž zahrnuty produkty 48hodinového klíčení – naklíčené semeno a klíček. Poslední experiment byl věnován srovnání vlivu různých způsobů vaření (konvenční, tlakové a za použití mikrovlného ohřevu) po různou dobu (vždy 4 časy) na stravitelnost bílkovin. Pro studium byly využívány základní chemické metody (obsah sušiny, tuku, hrubých bílkovin apod.), analýza aminokyselin (iontově-výměnná kapalinová chromatografie s postkolonovou ninhydrinovou detekcí), frakcionace proteinů na základné rozpustnosti v různých médiích a *in vitro* stravitelnost proteinů a sušiny.

Základní chemický rozbor ukázal, že testované luštěniny jsou bohatým zdrojem bílkovin (21.5-34.4 %) s vysokým obsahem albuminů. Ve zkoumaných luštěninách byly zjištěny následující relativní obsahy bílkovinných frakcí albuminů, globulinů, prolaminů, glutelinů a nerozpustného podílu: 40.3-48.5 %, 38.6-42.0 %, 3.5-5.3 %, 3.4-6.4 % a 2.8-7.2 %. Nejvyšší obsah aminokyselin v naklíčených semenech a klíčcích byl zjištěn u *P. sativum* (Xantos).

In vitro stravitelnost sušiny a proteinů se u syrových luštěnin pohybovala v intervalech 51.1-71.5 % a 54.1-75.0 %. *In vitro* stravitelnost proteinů se u naklíčených semen pohybovala nad 80 % a u klíčků nad 86 % (doba klíčení 48 hodin). Proces klíčení semen je jednoduchou metodou pro zlepšení stravitelnosti proteinů testovaných luštěnin.

Tlakové vaření a mikrovlnný ohřev (8-14 minut) po 6hodinovém máčení (0.2 % $NaHCO_3$) může být doporučen pro tepelnou úpravu testovaných luštěnin. Navrhované procesy zajistily dostatečnou stravitelnost proteinů. Z hlediska dostupnosti v rozvojových zemích lze doporučit zejména tlakové vaření.

Na základě zjištěných údajů vyplynulo, že některé odrůdy *P. sativum* mohou být úspěšnou alternativou *G. max*.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

Abbreviation	Meaning
CP	Crude protein
DM	Dry matter
EAA	Essential amino acids
FAO/WHO	Food and Agriculture organization
h	hour
min	minutes
n	Number of samples
NEAA	Non essential amino acids
NFE	Nitrogen free extract
IVPD	*In vitro* protein digestibility
IVDDM	*In vitro* digestibility of dry matter
TEAA	Total essential amino acids
TIA	Trypsin inhibitory activity
TNEAA	Total non-essential amino acids
TAA	Total amino acids
SD	Standard deviation

1. INTRODUCTION

Legumes are an annual crop, next to cereals, belongs to the family of *Leguminosea*. They are a good and inexpensive source of dietary proteins, carbohydrates, vitamins and minerals [1]. Occurrences of malnutrition have increased in developing countries, with the increase in population and inadequate supply of protein [2]. This is mainly due to the consumption of cereal-based porridge which is bulky, with low energy, low nutrient density and high anti-nutrient content [3]. In this context, legumes can play an important role to offset this trend due to their high content of proteins ranging from 20-40% [4]. Since plants provide 70% of the world supply of protein on a global basis [5], legumes can provide a significant contribution to the protein requirement of human diet.

Apart from that, legumes are recognized as a prestige food item in the diets of developed countries due to health problems resulting in consumption of meat, as well as the discovery of the benefits of legumes in the diet and the protection they afford against colon disease [6]. Accordingly, in developed countries, plant proteins are now regarded as either versatile functional ingredients or as biologically active components, rather than as essential nutrients. This evolution towards health and functionality, which is mainly driven by the partial replacement of animal foods with legumes, has shown to improve nutritional status [7] due to low cholesterol level in plant foods, and increased level of fibre intake which reduces the risk of bowel diseases, including cancer [8].

The nutritive value of a protein depends on its composition, digestibility and bioavailability of essential amino acids. Many studies on proteins of legumes, explain different reasons for limited digestibility of the seeds, such as the type of proteins present in the legume, its limited susceptibility to hydrolysis by digestive proteases, due to its structural characteristics [9] and certain antinutritional factors. From the nutrition point of view, only thermally processed pulses are important since pulses are never eaten raw. A factor that is attributed for the less use of legumes is it being hard-to-cook. A variety of processing methods have been practiced such as soaking, germination and cooking. Many investigators have found out that prolonged soaking and cooking periods were required to soften the legumes which had been stored for long periods of time.

Recently there is an interest in germination, because it is a natural process, with minimal energy and technical requirements. Therefore, its use is easy. At the same time it was noted that it increases the nutritive value of the seeds [10,11] as a healthy [12], as minimally processed and additive-free nutritional foods. Therefore, eating of germinated legumes is beneficial to health.

Soaking legumes in water before cooking is a common practise. However, it takes a long time to cook. Soaking in $NaHCO_3$ solution and cooking was found

as a better alternative to conventional processing methods and it improves the protein digestibility for some legumes [13] and greatly influenced their nutritive values as well. However, the effect of different concentration of $NaHCO_3$ on cooking time for all the legumes was not studied. Method of cooking also plays an important role as it influences the bioavailability and utilization of nutrients and also improves palatability that incidentally may result in enhancing the digestibility and nutritive value [14].

It has been reported that soybeans have had a competitive advantage over other legume seeds [15]. The main protein source for animal feed in Europe is soybean [16] which is imported, causing a high expenditure on feed for animal production in Czech Republic [17]. Further, Hochman stated that animal production has declined due to insufficient animal feed. Hence, the search for an alternative to soybean, as sources of protein, is important. This alternative should already be widely grown in temperate countries.

Among the legumes, pea (*Pisum sativum* L.) is a crop widely grown in temperate countries including Czech Republic though the consumption is low when compared to other sources of proteins. Peas are more adapted to European cropping conditions than soya bean. More than 3 million tons of peas were produced in European community in (2004) and 85% was used in animal feed [18]. Further, it has been reported that feed peas are an excellent source of nutrients for all species of animal with high digestibility and palatability [19].

Much research was conducted to optimize soaking and cooking treatments of legumes. However, studies on quality of protein, by evaluating digestibility for different cooking methods and cooking intervals, after soaking in $NaHCO_3$, for varieties of peas widely grown in Czech Republic and Slovak Republic is minimal.

Moreover, though many studies have been carried out on germinated legumes there is lack of information on nutritive value and digestibility of cotyledons and radicles separately after germination. There is limited information on characterization, protein fractionations, of legumes grown in Central Europe.

Therefore, it is important to conduct more in-depth studies of nutritional quality of low cost protein sources such as legumes grown in Central Europe and investigate the effect of different cooking methods on protein and its digestibility.

The underlying philosophy of this dissertation is primarily to find ways to make the best use of legumes to fulfil nutritional needs of the global population as well as its use for animal feed.

2. SCOPE OF THE STUDY

The scope of this study is to perform such investigations, in keeping with the underlying philosophy, namely, to find ways to utilize legumes effectively and to introduce such legumes widely grown in Central Europe as an alternative to soya bean to alleviate the problem of protein energy malnutrition, fulfilling the nutritional needs of the global population as well as its use for animal feed. In order to fulfil this aim the following objectives were formulated.

1. To investigate the nutritional composition by analyzing, dry matter, ash, crude protein, crude fat, crude fibre, nitrogen free extract, organic matter, amino acids, protein fractions, *in vitro* digestibility of crude protein and dry matter with the view of characterization raw legumes.
2. To study the enhancement nutritive value of legume seeds by developing simple processing techniques, such as germination of legumes, by analyzing the dry matter, crude protein, amino acid, *in vitro* digestibility of crude protein and dry matter in cotyledons and radicles separately after germinating for 48 h.
3. To investigate the use of $NaHCO_3$ solution as a soaking medium instead of water with the view to reduce soaking and cooking time.
4. To study the effect of normal cooking, pressure cooking and microwave cooking with different time intervals on *in vitro* digestibility of crude protein in legumes with the view to identify and introduce the most appropriate cooking method and time that retains optimum amount of proteins and highest digestibility.
5. To find the possibility of using pea (*P. sativum*) which is mainly grown in Central Europe to use as an alternative for soya bean as a feed for both human and animals and thereby introduce the pea as effective substitute for soya bean.

3. LITERATURE REVIEW

3.1 Legumes as a world food crop

Legumes are the most important plant species in the family of *Leguminosae*. They are second only to cereals in providing food crops for world agriculture [20]. Legumes are recognized as a crop that plays an important role in the agriculture, as well as a major source of dietary nutrients for many people in the developing countries. According to the FAO estimations the world production of legume seeds, was about 58 million tons in 1994 and of this, the major part, was produced by developing countries [2]. According to FAO data, a summary of distribution and production of legumes are shown in the table below.

Table 1. Worldwide distribution (%) of grain legumes seed production

Land	Total area (%)	Production (Million Tons)	Production (%)
Africa	26.2	8	14.5
North America	8.3	8	14.5
South America	7.6	4	7.3
Asia	50.0	26	46.8
Europe	5.3	7	13.4
Australasia	2.6	2	3.5
World	100.0	55	100.0

Sources: calculated from FAO data (2000) [21]

3.1.1 Nutritional importance of legumes

Legumes occupy an important place among the food crops due their high nutritional value. Awareness on nutritive value and health benefits of legumes is of vital importance with the objective to increase consumption of legumes in daily diet of the human.

Protein content of legumes is ranging from 20 to 40% of their dry matter [4]. In most of legumes fat content is relatively small (<1% of their dry matter) [22]. A few species such as soya, ground nut and lupin have higher amount of fat (5-20% in range). Dietary fiber fraction plus total starch and soluble sugars, ranged from 59 to 67% of the dry matter [22]. The B-vitamin is mainly found in legumes and these contents increase with germination [23]. It is a very good

source of folates [24]. High levels of both α- and γ- tocopherol (8.2 and 7.9 mg per 100g, respectively) were found in peanuts [25]. According to a study on a 100g dry weight basis of some commercial bean varieties, the mean vitamin values of thiamin 0.99 mg, riboflavin 0.20mg, niacin 1.99 mg, pyridoxine 0.49 mg, folic acid 0.30 mg. Retention of water-soluble vitamins during cooking averaged between 70 and 75% [26]. Vitamin A and vitamin C content of legumes vary depending on the species. They range from 12-90 μg of beta carotene per 100g and 1-5 mg per 100g of legume seeds respectively for commonly consumed legumes [27]. Generally minerals content of legumes were found as 3-4% of their dry matter [22] and mean values minerals were found for phosphorus 0.46 g, sodium 10.3mg, potassium 1.5 g, calcium 0.2 g, magnesium 0.2 g, zinc 3.2 mg, manganese 1.4 mg, copper 0.9 mg, and iron 5.84 mg. Retention of minerals during cooking ranged from a low of 38.5% for sodium to total retention for calcium [26]. Bravo et al. [22] have reported that black gram as the legume with the darkest seed coat, had the highest polyphenolic content (3.98% of their dry matter). Since the discovery of the role of polyphenols as anti-oxidants [28], it is also can be considered an important nutrient.

3.1.2 Health Benefits of legumes

Legume proteins contribute energy and amino acids, which are essential for growth and maintenance. According to Liao et al. [29], consumption of legumes proteins could reduce plasma low density lipoproteins and help in reducing weight. Singh et al. [30] have reviewed that soy protein lowers blood cholesterol.

Apart from being a valuable source of protein, consumption of legumes has also been linked to reduced risk of diabetes and obesity, coronary heart disease [31], colon cancer, and gastrointestinal disorders. Legume starch causes less changes in plasma glucose and insulin upon ingestion [32] as it has identified as low glycaemic index [33] and therefore, it is a very good source of nourishment for controlling diabetics.

Consumption of legumes may also have a protective effect against prostate cancer in humans. The phenolic compounds present in these legumes are known to exhibit strong antioxidant, anti-mutagenic, and anti-genotoxic activities [11, 34].

Further, isoflavones, a class of phytochemicals found in soybeans contributes towards cancer prevention. Soybean is an alternative source of protein for people who are allergic to milk protein and also highly digestible (92 to 100%). It contains all essential amino acids. Although relatively low in methionine, it is a good source of lysine. Soy-protein products contain a high concentration of isoflavones, up to 1g/kg [30].

Since health and nutrition is the most demanding and challenging field, legumes can play an important role, not only to the dietary pattern of low-income groups of people in developing countries, but also for the people in rest of the world as it has lot of health benefits. The contribution of legumes towards a global healthy population therefore cannot be underscored.

3.1.3 Factors affecting the nutritional quality of legumes and remedies

Despite of high nutritional value in legumes, they contain several anti-nutritional compounds as well, such as, trypsin and chymotrypsin inhibitors, lectins, tannins, phytic acids and amylase inhibitors [5]. In order to improve the nutritional value and to provide effective utilization of legumes to a maximal level, it is essential to remove activity of anti-nutritional factors. In this regard, many researchers have shown that these anti-nutritional factors can be eliminated by different processing methods [9,30,35].

Table 2. Methods of processing found to reduce anti nutrients in legumes by past authors

Methods of processing	Conditions	Legume	Anti-nutrients eliminated	References
Cooking	Combine with soaking	Peas cultivars grown in New-Zealand	Trypsin inhibitors average reduction of 78%	[35]
		Soya bean	Phytic acids	[36]
Heat treatment	at 50°C for 2 h.	Cowpea	Polyphenols, tannins	[9]
		Soya bean	Phytic acids	[30]
Soaking	Water pH (around 5.5)	Soya bean	Phytic acids	[36]
Germination		Soya bean	Phytic acids	[36]
Extrusion cooking	Temperature (100-140°C, 148°C, 25% moisture 100 rpm	Pinto bean *Phaseolus vulgaris* L.)	Trypsin, Chymotrypsin inhibitors	[37]
		Pea	Amylase inhibitor, Tannins	[38]

Combined soaking and boiling is found as an effective method of reducing most of anti nutritional factors [35]. It is observed that a significant reduction of most of all anti nutritional in legumes takes place upon cooking at temperature ranges from 50-100°C for 30 min-2 h [9,39**].** Steaming processes at desired pressure (34.5 and 103.5 kPa) cause smaller losses in total phenolic compounds.

Though polyphenols were traditionally considered as anti-nutrients, this view has changed in the last few years with the discovery of the role of polyphenols as anti-oxidants and anti-mutagens [28]. Therefore, steaming retains more anti-oxidants than the boiling processes [28]. Extrusion cooking at temperature ranges from 100-148°C is the best way to remove anti-nutritional factors in legumes, for processing in commercial scale, permitting consumption without any health risk factors and for well being not only for human but also for animal feed as well.

3.2 Important food legumes in Central Europe

3.2.1 *Pisum sativum*

Pea (*Pisum sativum*) is a widely availed legume, usually produced in temperate regions, but use as an important food legume through out the world [40]. Annual production of pea is 4-5 tons of seed per hectare with 230-280g CP/kg of DM [41]. Pea is the main protein crop cultivated in the EU. In 2004 the area sown in the EU-15 was 790,000 ha. France is the largest producer (60% of EU production), well ahead of Germany and the United Kingdom [42]. Further, peas are now considered to be an important source of proteins for animal feed [18] as well as a possible raw material processed for human food in European countries [43].

This is a self pollinated annual herb, bushy or climbing, stems being weak, round, and slender, 30-150 cm long; leaves alternate, pinnate with 1-3 pairs of leaflets and a terminal branched tendril leaflets ovate or elliptic, 1.5-6 cm long [44].

Pea probably originated in south western Asia and thereafter spread to the temperate zones of Europe and reported to be originally cultivated as a winter annual crop in the Mediterranean region [44].

The ideal mean temperature for growth of pea is 55-65°F (13-18°C) [45].

The protein content of pea seeds appears highly variable and is influenced by both genetic and environmental factors [43]. Peas are good sources of dietary carbohydrates, like many food legumes [46]. Dry peas contain large amounts of oligosaccharides and polysaccharides [40].

Pea lipids of ether extractives are normally less than 20 g/kg seed DM, but can range up to 60 g/kg and contain high proportion of unsaturated fatty acids, notably linoleic [40]. Further, it consists of mineral elements relatively richer in calcium and potassium [40].

As the utilization of soyabean meal is increasing in Europe, peas are an alternative protein for monogastric animals. They offer a bulk source of seed

protein for man and animals from a relatively short growing season compared with other legumes [47].

Pea protein concentrates and isolates are commercially available and are valuable functional ingredients widely used in food formulations [48]. Pea flours, concentrates and isolates have been suggested as alternative protein sources for several food products [49].

Pisum sativum* var. *arvense

Field pea (*Pisum sativum* var. *arvense*) is a more vigorous form of *P. sativum* cultivated in the Mediterranean rainfall area of South Africa [50]. Less sweet seeds which are usually eaten as a protein crop when they are mature.

This is an annual crop grown to height of 2 m. It flowers from May to September and the seeds ripen from July to October.

The plant grows well in light (sandy) and medium (loamy) soils and requires well-drained soil. The plant prefers neutral, basic (alkaline) soils and moist soils. It cannot grow in the shade [51].

Cooked or sprouted seeds are good source of protein. They are grown mainly for use when mature and dried.

3.2.2 *Glycine max*

Soya bean (*Glycine max)* is presently grown at a rate of 155 million metric tons per year worldwide. Seed legumes provide one-fifth of all plant proteins consumed by man on a global basis [52]. Use of soya bean meal is increasing in Europe [18].

Soya bean originates from Asia but the United States produces 38% of the total soybean crop in the world, followed by Brazil (25%), Argentina (19%), China (7%), India (3%), Canada (2%), and Paraguay (2%), while all other countries grow only about 4% [30].

This is an annual plant, with an erect and ramified stem, the height varies from 0.45 m to 1.5 m, alternate trifoliate leaves, and axillary clusters of flowers. The flowers are red or violet. The beans grow in pods that develop in clusters with each pod containing 3 to 5 beans. A soybean plant produces 60 to 80 pods. The beans are round or oval. The colour of the beans can vary from yellow, green, brown, violet, or even black with white spots [30].

The optimal temperature for growth is 30°C. Soya is very sensitive to photoperiod and most cultivars will only bloom when day length is less than 14 hours, it will also not set seed if night temperatures fall below 10°C. Very short days will lead to premature flowering producing small plants and reduce yields. [53].

It has been noted that soybean seeds have a protein content of 35-45% on a dry weight basis [54]. About 18% of the beans consist of oil (0.5% lecithin), which is rich in polyunsaturated fatty acids (54% linoleic acid, 22% oleic acid, and 7.5% linolenic acid) and contains no cholesterol. The rest of the beans consists of moisture (14%), soluble carbohydrate (15%, sucrose, stachyose, raffinose, others), and insoluble carbohydrate or dietary fiber (15%) [30]. Soybeans have been transformed into various forms of soy foods, tofu being the one most widely accepted throughout the world [55]. It has been estimated that approximately 60% of processed foods contain ingredients that are derived from soybeans [54].

3.2.3 *Lupinus albus*

White lupin (*Lupinus albus*) is an economically and agriculturally valuable plant which is able to grow in different soils and climates. Interest in lupin production is increasing because of its use as a source of protein, for pharmaceutical purposes or as a green manure. It has high alkaloid content, which is a natural component of plant pesticides [56].

The white lupin is an old world species mainly distributed around the Mediterranean and along the Nile valley. The white lupin is sometimes cultivated, especially in South Europe, for its edible seed and also as a green manure crop [57].

This is an annual crop growing up to 1.2m by 0.25m. It flowers from June to July, and the seeds ripen from August to September.

The sweet varieties are perfectly wholesome as food for humans [51]. Seeds of white lupin have a protein content ranging from 33 to 47% according to genotype and location, with a slight deficiency in sulfur amino acids and lysine compared to the FAO/WHO standard for dietary protein [58]. Oil content varies from 6 to 13% with a high concentration of polyunsaturated fatty acids [57].

Lupin seeds may also be a potential source of alimentary cellulose for the production of dietetic food. The high protein fraction could be used as a substance for enriching different kinds of products, such as pastries, breads, chips and milk substitutes and also be a main food component when animal proteins are eliminated [56].

3.2.4 *Faba vulgaris*

Broad bean (*Faba vulgaris)* seeds are very nutritious and are frequently used as items of food.

This is an annual crop grown to 1m height at a fast rate. It is not frost tender. It flowers from May to August, and the seeds ripen from July to September.

The largest producer of faba bean is China, followed by Ethiopia and Egypt [59].

The plants grow well in light (sandy), medium (loamy) and heavy (clay) soils that require well-drained soil and can grow in heavy clay soil. It requires moist soil, though can tolerate drought and prefers acid, neutral and basic (alkaline) soils. It can grow in semi-shade (light woodland) or no shade. The ideal temperature ranging between 18 and 27°C in the growing season and pH ranging from 5.5 to 7 [51].

The immature seeds can be eaten raw when they are small and tender, as they grow older they can be cooked as a vegetable. They have a very pleasant floury taste. Mature seeds can be eaten cooked as a vegetable or added to soups etc. They are best soaked for 12-24 h prior to cooking in order to soften them and reduce the cooking time. They can also be dried and ground into flour for use in making bread etc with cereal flours. The seed can also be fermented to make 'temphe'. The seed can be sprouted before being cooked [51].

3.3 Major proteins and amino acids in legumes

Legumes are recognized as a rich source of proteins in plant kingdom because they have large amount of protein contents, ranging from 20 to 40% of their dry matter, according to species, genotypes within species and environments. The proteins in legume seed mainly store in the cotyledonary tissues little in embroyonic axis and testas as those represent small proportions of the seed mass [4]. Proteins in legume seeds represent from 20% (dry weight) in pea and bean upto 38-40% in soya bean and lupin [20]. Many studies on legume proteins explain different reasons for limited digestibility of the whole seeds. One of the main factors affecting their limited digestibility is kind of proteins present in the legume and its limited susceptibility to hydrolysis by digestive proteases due to structural characteristics [60].

Proteins in legumes are usually classified into two major fractions: globulins and albumins. Further, prolamin and glutamine fractions have noted as very low amounts [8]. Many authors have reported that Osborne classified protein in to groups on the basis of their extraction and solubility in water (albumins), dilute saline (globulins), alcohol/water mixtures (prolamins), and dilute acid or alkali (glutelins). It has been further explained that globulin are salt soluble, albumins are water soluble [20], prolamins which are soluble in ethanol/water solutions [20] and glutalins which are soluble in sodium hydroxide [61].

Types of proteins in legumes are vary and it may be depend on species, cultivar etc. Albumin was found to be the major protein in some cultivars of *P. sativum* (cv. ucero, cv. ramrod and cv. agra) [62]. How ever, some authors have shown that the majority of proteins in legume seeds are the globulins usually account for about 70% of the total protein and glutelins (10-20%) and albumins

(10-20%). The globulins in most legumes are in two classes as legumins (11S) and vicilin (7S) [4,63,64]. Globulins comprise the bulk of the proteins in lupin seeds [65].

Legumes have all EAA and NEAA in their amino acid profiles as shown in appendix A. However, some are unbalanced. When compared to egg protein, the indispensable sulphur-containing amino acids are at a much lower concentration. Sulphur containing amino acids (i.e. methionine and cystine) are considered as the most critical limiting components of the proteins. The various legume protein sources may differ significantly in the amino acid composition [4,66,67]. However, when compared to soya protein lysine content is higher in other legumes [4]. The albumin fraction contained the highest amount of total sulphur-containing amino acids followed by glutelin, globulin and prolamine in beach pea [61]. All legume species were rich in aspartic acid/ asparagine and glutamic acid/glutamine that impart acidic character to legume proteins [66] [68]. Legumes are found as a cheap source of lysine and it is very important for successful feeding for increasing population [67].

3.3.1 Structure and functions of proteins and amino acids

Proteins are composed of 20 basic units called amino acids (Appendix A) which consist of a central carbon atom (the alpha-carbon) bound to an amino group (NH_2), a carboxyl group (COOH), a hydrogen atom, and one of 20 different R groups. The alpha-carboxyl group of one amino acid is joined to the alpha-amino group of the next by an amide bond (also called a peptide bond) to form chains of amino acid residues (polypeptide chains) Figure 1 [69].

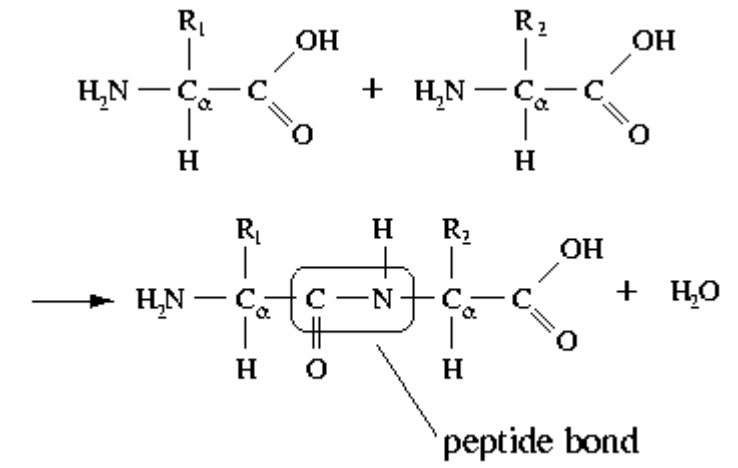

Figure 1. Peptide bond linking two amino acids [69]

Any number of amino acids can be joined together to form peptides of any length. A protein is a naturally occurring polypeptide with a definite 3-dimensional structure and that is unique to each protein.

Protein structure is organized hierarchically from so-called primary structure (Figure 2) to quaternary structure (appendix B).

Specifically, primary structure is a complete description of the covalent bonds of a protein with the exception of disulfide bonds. In contrast, the higher orders of proteins structure (i.e. secondary, tertiary and quartenary) involve mainly non-covalent interactions.

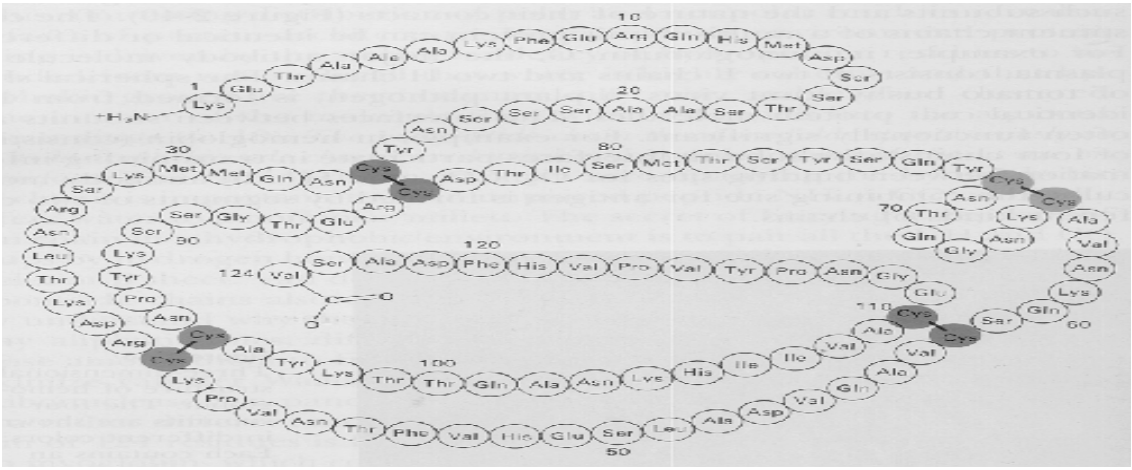

Figure 2. Primary structure of a protein (eg. Ribonuclease)
Source: (Spriggs) [69]

The secondary structure of a protein is the 3D arrangement of amino acid residues that are relatively near one another in the linear sequence. Secondary structure is created by hydrogen bonding between the alpha-amide groups and alpha-carbonyl groups of the backbone, to enable globular proteins to retain a minimum energy conformation. The commonest secondary structure elements in proteins are the alpha (a) helix and the beta (b) sheet (sometime called b pleated sheet).

The tertiary structure of a protein is a description of the way the whole chain (including the secondary structures) folds itself into its final 3-dimensional shape (Appendix B)

Quaternary structure involves the association of two or more polypeptide chains into a multi-subunit structure [69].

Proteins play very important roles in nearly all biological processes and display a wide variety of functions both within the cell and as well as extra-cellular. For example, the majority of chemical reactions occurring in the cells of living organisms require protein catalysts called enzymes. Proteins are also involved in the transport of molecules (within cells and out of cells), in the recognition of other cells, in the coordinated motion seen in muscle fibres, the excitability of nerve cells, and in the control of cell growth and differentiation.

The surface of each protein has a unique chemical reactivity determined by the amino acid residues exposed and their exact orientation to each other. The

many different structures that proteins can adopt are therefore responsible for the vast array of functions that proteins exhibit, and for allowing the binding of so many different types of ligand. Ligands that are bound by the protein must fit snugly into the shape of the protein at the binding site (active site) to allow the weak interactions that hold them [69].

3.3.2 Methods and problems of amino acids analysis of legumes

Legumes are a valuable source of plant proteins. In many food applications, such as processing, fortification, optimum diet formulation and food composition data base, exact quantities of protein in legumes are required to be determined. Among the techniques used, amino acid analysis has been recognized as an exact method to measure the protein content in foodstuff by quantifying each of the amino acids and summing the values.

Amino acid determination of protein is relatively complex analytical process, consisting of two steps, complete hydrolysis of the substrate to liberate the residues, followed especially by chromatographic analysis and quantification of the liberated amino acids [70]. Amino acids can be hydrolysed by using acids, alkaline or enzymes [71].

Although the main objective of hydrolysing is to quantitative liberate all amino acids of the substrate and quantitative recovery of them in the hydrolysate, no hydrolysed method has been found to liberate all amino acids simultaneously. Since several factors affect on hydrolysis such as temperature, time, hydrolyzing agent [70] some amino acids and cannot be quantified. Albin et al. [72] have noted that amino acid concentration of sample affected by concentration of acid use for hydrolysis.

Further, according to the findings, cystine partially destroys, methionin partially oxidizes and tryptophan completely destroys during acid hydrolysis. Also asparagine and glutamine are converted to aspartic acid and glutamic acid respectively during acid hydrolysis they can be determined by acid hydrolyse [73]. Due to these reasons cystine and methionin are determined after oxidizing with performic acid (mixture of H_2O_2 and HCOOH in volume of 1: 9) for 16 h at 0°C prior to hydrolysis and are measured as cystic acid and methionin sulphone. Wathelet, [74] has noted in his research that, if the sample is oxidized before acid hydrolysis step, tyrosine content estimation will not be accurate.

The most critical step in the analysis is hydrolysis of protein in the sample. In legumes this hydrolysis is usually performed with 6M HCl, heated at a temperature selected between 105-110°C in a time range from 16 to 70 h, depending on type of source. However, according to many literature, 6M HCl was used as the hydrolyzing agent in acid hydrolysis and hydrolysis was carried out in a temperature range of 105-110°C for 21-24 h [75,76,77,78]. Then HCl was subsequently removed under vaccum and that ropy sample was

reconstituted in sodium-citrate buffer at pH 2.2 [76]. After filtering the hydrolysed sample, it was used to determine amino acid content by different automated amino acid analysers especially based on ion exchanged separation [[75,76].

Tryptophan is determined after alkali (NaOH) hydrolysis by a colorimetric method [75]. Ravindran et al. [71] has reported that use of NaOH as hydrolyzing agent appears to offer advantages over the use of $Ba(OH)_2$ or LiOH, as the precipitation/adsorption problems associated with $Ba(OH)_2$ and solubility problems with LiOH that do not occur with the NaOH.

Ion exchange chromatography with post column ninhydrin detection with Na-based cat-ion exchange system is widely used method to quantify the amino acids of legumes in the research carried out by many countries. Amino acids are separated on ion exchange column through pH and cat-ion strength. A temperature gradient often employed to enhance the separation. When amino acids reacts with ninhydrin the reactants have characteristic purple colour (Figure 3.) and show maximum absorption at 570 nm except amino acids such as proline which gives yellow colour and it gives maximum absorption at 440 nm. Accordingly, the post column reaction between ninhydrin and amino acid eluted from column is monitored at 440 and 570 nm and the chromatogram obtained is used for the determination of amino acid composition [79].

However, precolumn derivatization with o-phthaldialdehyde using reverse-phase high-performance liquid chromatography (HPLC) was also used to quantify the amino acids of legumes by some of the investigators [80,81,82] used phenylisothiocyanate, for derivatization of amino acids in fermented soya bean.

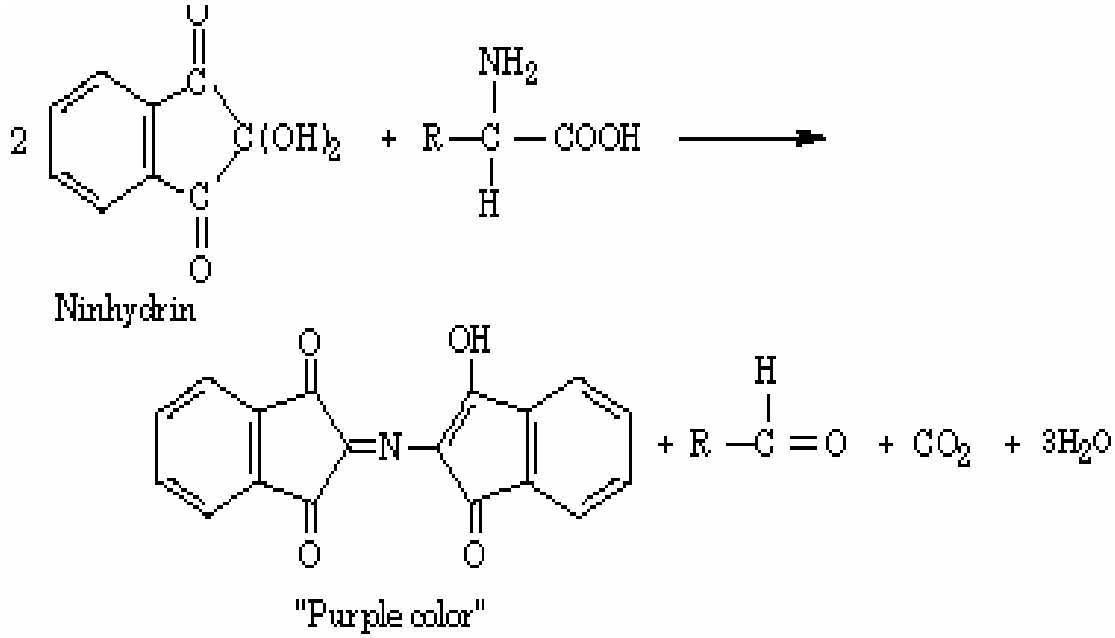

Figure 3. Reaction of amino acid with ninhydrin

3.4 Germination of legumes

Germination is a biotechnological process in which metabolic enzymes, such as proteinases are activated and thereby, release some amino acids and peptides. This may use to synthesis and utilization to form new proteins. As a consequence, the nutritional quality of proteins is enhanced and therefore, germination has been identified as a simple technological procedure for improving the nutritional quality of legumes [83,84,85].

According to the methods widely used for germinating legumes by past investigators, chick peas were germinated after soaking the seeds at room temperature in de-ionised water for 18 h, in sterile petri-dishes lined with wet cotton wool for the respective number of days [86]. Another author have noted that after soaking chick pea seeds in tap water for 24 h at room temperature, spread evenly on a tray lined with absorbent paper and then placed in a controlled environment chamber at 28°C. They were washed twice a day to avoid microbial growth. Tap water was sprayed throughout the germination period thrice daily [11]. With regard to pea, beans and lentils, seeds were soaked in (1:5 w/v) water containing 0.07% sodium hypochlorite (NaOCl) at room temperature for 30 min. The seeds were then drained, rinsed in pH-neutral water, and soaked in distilled water for 5½ hours. Then seeds were germinated at pilot scale by layering them over moist filter paper continuously watered by capillary action in a germinator (G-120 Snijders, Holland) at 20°C temperature and 99% relative humidity for 2,4,or 6 days with light and without light during the whole germination period [10].

3.4.1 Benefit of germinating legume seeds

Interest in germination of legumes has grown recently because it is a natural process, with minimal energy and technical requirements and due to the increase of nutritive value of the seeds [10,11,85]. Further, sprouts have gained popularity in Western countries as a traditional, biotechnological, healthy food [12] and they are also used to produce flours of high nutritional value [1] to the human.

More over, germination has ability to decrease levels of anti-nutritional factors present in legume seeds, at the same time improve the concentration and bioavailability of their nutrients [87,88] when compared to raw seeds. The extensive breakdown of seed-storage proteins that takes place during this process improves protein digestibility and the essential amino acid content, thus enhancing the nutritional value of legumes [1,89]. Further, as fats and carbohydrates that often at surplus levels in Western diets are broken down while dietary fibre, increases in germination [1].

Due to the tenderness of sprouted part of germinated seeds, it may not be required to cook long time as raw seeds.

3.4.2 Conditions of germination affecting nutrients and anti nutrients

Different authors have shown that the germination process increases the protein content of legumes although other authors have observed no changes or lower protein content in sprouts, and results seem to depend, not only on the seed cultivar, but also on the germination conditions (temperature, light, time) [90,83].

The growth conditions during the germination process can have important effects on the composition of secondary metabolites of nutritional importance [1].

As germination progressed, CP and nitrogen free extractives (crude carbohydrate) decreased gradually, whereas fat, crude fibre, ash and food energy increased in *Cajanus cajan* [86]. How ever, some authors have noted that significant increase in CP, non-protein nitrogen and crude fiber in germination of chickpea seeds when compared to the raw chickpea seeds. This increase was mainly due to the use of seed components and degradation of protein to simple peptides during the germination process [91]. Some of the investigators reported an increase in protein content after 3 and 4 days germination for chickpea and faba beans, respectively [92].

Germinated chickpea seeds showed noticeable decrease in the contents of K, Ca, Mg, Mn and Cu. However, germination increased the Fe, P and Zn contents by 2.46, 5.75 and 14.12%, respectively [91]. Germination of the *Cajanus cajan* seed for up to 4 days resulted in significantly higher contents of iron, calcium, magnesium and phosphorus [86].

Considering the functionality of the nutrients, it has been reported that long germination periods have a negative effect on the organoleptic properties of legume seeds. It has been reported that germination for periods exceeding 48 h produces considerable losses of dry matter through respiration [93]. With regard to peas, 2 days of germination would be sufficient to significantly improve the palatability and nutritive utilization of protein and carbohydrates from *P. sativum*. The presence or absence of light during the germination process did not affect the nutritive content [87].

The amino acids of bean, histidine, glutamate, glycine, histidine, arginine, tyrosine and tryptophan contents were declined after germinating, while asparagine, valine, isoleucine, phenylalanine and tryptophan contents were varied in different ways, depending on the germination conditions. Germination of lentils produced an increase of free protein amino acids (FPAs) and the appearance of new ones. The same effect was observed in peas, but histidine disappeared and the changes of aspartic acid, asparagine and arginine contents were dependant on the germination conditions [1].

Germination significantly reduced both thiamin and niacin. However, a significant increase in riboflavin and pyridoxine in chick pea was noted and the

retentions of pyridoxine and riboflavin were 103.58 and 116.15%, respectively. Further, the stability and retention of B-vitamins in germinated chickpea seeds was noted as higher than in cooked seeds [91].

Germination was more effective in the reduction of oligosaccharides, especially raffinose and stachyose which causes flatulence and phytic acid than cooking treatments [11,91]. The higher reduction of phytic acid could have been due to the phytase activity during germination [91].

Based on the studies of IVPD of *Dolichos lablab* cultivar mani avare, had high protein digestiblity in the initial stages of germination was reported [14]. How ever, a literature search failed to find any studies dealing with variations in the TAA with germination time in the legumes considered. The only values reported were for samples at the end of germination, and there was substantial variability in the results [10]. There are some reports about the effect of germination on the nutrient and antinutrient contents of some legumes, such as soybeans, mung beans or lentils, but very little information is available for peas and beans [1].

3.5 Soaking of legumes

Soaking legumes in water for at least a few hours (preferably overnight) before cooking is a common practice performed to reduce cooking time and thereby becomes much easier to cook [91]. Further**,** soaking has been documented to be an effective treatment to remove anti-nutritional factors and improve IVPD, but the effects varied with legume cultivars, soaking conditions such as type of soaking solutions, soaking period and temperature [94].

According to the studies on soaking of legumes by past authors, different method of soaking for different legumes, i.e., soak in deionized water (1:5 w/v) at room temperature for peas [95], in distilled water (1:5 w/v) at ambient temperature for 12 h for beans [5], distilled water (1:5 w/v) at 30°C for 16 h for faba beans (*Vicia faba*), pea seeds (*P. sativum*), chickpeas (*Cicer arietinum*) kidney beans (*Phaseolus vulgaris*) [96], water 1:2 w/v and 16 h for pea, common bean, chickpea and lentil legumes [95], distilled water 1:10 w/v, at room temperature (25°C) for 12 h for mung beans [98] were used.

The addition of salts to the soaking or cooking water of pulses is often used to reduce the cooking time. $NaHCO_3$ is the most commonly used and it always use in Egypt as a traditional practices in treating these legumes [94]. Legumes in India were soaked in distilled water or 0.02% (w/v) $NaHCO_3$ solution (pH 8.6) for 2, 4 and 6 h in a bean:water ratio of 1:10 (w/v) [13]. Soaking of horse gram (*Dolichos biflorus*) in a solution of 1.5% $NaHCO_3$, 0.5% Na_2CO_3, and 0.75% citric acid for 12 h and suitability of this treatment for reducing cooking time of several other legumes was evaluated before [99].

3.5.1 Effect of soaking in distilled water

Soaking in water for 16 h is recommended for improvement of nutritive value of peas, chickpeas, faba and kidney beans. This enhances the utilization by human and animals. In some cases decrease of protein levels was noted when soaking in distilled water and it could be due to leaching of some of the water-soluble proteins into the soaking medium [96].

Since polyphenolic compounds are water-soluble in nature and mostly located in the seed coat, the decrease in the level of phenolics and tannins during soaking may be attributed to leaching into the soaking medium. Even though the soaking of seeds for 6 h in distilled water is effective in reducing significant levels of tannins and phytic acid content, it does not cause any improvement in the protein digestibility of *B. purpurea* seeds, which is in agreement with an earlier study of *Vigna aconitifolia*. In some cases like *Prosopis chilensis* and *Dolichos lablab* var. *vulgaris,* an improvement in the IVPD (3%) was recorded. Although oligosaccharides are water-soluble, soaking the seeds in distilled water resulted in a limited reduction in the flatulence factors, regardless of the soaking time [13].

3.5.2 Effect of Soaking in $NaHCO_3$

Authors have observed that use of different solutions have an effect on reducing cooking time. Soaking of horse gram (*Dolichos biflorus*) in a solution of 1.5% $NaHCO_3$, 0.5% Na_2CO_3, and 0.75% citric acid for 12 h was found to be effective in reducing cooking time from 145 to 27 min. The treatment improved protein digestibility of cooked horse gram from 69 to 78%. Horse gram cooked after pre-treatment with soak solution had 35% less amount of polyphenols than that in untreated cooked samples. The suitability of this treatment for reducing cooking time of several other legumes was evaluated [99].

There are large variations in seed characteristics among the different types of legumes which in turn influence on the soaking and cooking characteristics of seeds [100]. According to the hydration coefficient reported after soaking with 0.5% $NaHCO_3$ for soya, bean, and lupin, it is noted that it increased its rate in the first 6 hours of soaking and became steady after 9 h, 7 h and 8 h respectively and 0.5% $NaHCO_3$ led to decrease of flatulent inducing components, anti-nutritional factors, non protein nitrogen, total carbohydrates, starch, reducing sugars, minerals (except Na) and protein solubility. Total protein, ash, fat, fiber, available lysine and *in vitro* protein digestibility was increased [94]. The reduction in oligosaccharides content during the soaking of the seeds in $NaHCO_3$ solution is slight but higher than that of soaking in distilled water. The loss of raffinose (19%), stachyose (15%) and verbascose (26%) was observed when soaking the seeds for 6 h and it may partly be due to leaching into the

medium because of change in the permeability of the seed coat caused by the ionic strength of the soaking medium [13].

Soaking in $NaHCO_3$ solution is effective in significantly reducing levels of total free phenolic and tannin content, compared to other processing methods and it improves the protein digestibility of *B. purpurea* seeds by 6% [13].

3.6 Cooking of legumes

Legumes have been recognized as "hard-to-cook" and this could be a particular factor that discourages the use of legumes. In general cooking is carried out for legumes after soaking. Cooking of legumes in boiling water is the most common method used to obtain a palatable product with improved nutritional value, digestible and to deactivate anti-nutritional factors.

Appreciable research efforts have been devoted to optimize soaking and cooking treatments of legumes and it has been noted that excessive cooking, however, can result in decreasing nutritive value [101].

During the cooking of legume seeds, two simultaneous processes occur inside and outside the cotyledon cells. Gelatinization of intracellular starch and de-naturation of proteins causes softening of the seeds. These result in the plasticization or partial solubilization of the middle lamella, leading to the separation of individual cotyledon cells [100]. The common subjective method to measure softness of the cooked seeds is by squeezing the seed between the fingers. The objective methods for determination of the optimum cooking time were: Measuring the time required to penetrate the cooked seed by a plunger of defined weight, measurement of the force required to compress or extrude cooked seeds through an extrusion grid and measurement of the force required to shear cooked seeds using a multi-blade shear press [100].

Since ordinary cooking is time consuming, pressure cooking, microwave cooking are commonly practiced for legumes in domestic scale while the extrusion cooking is carried out for in commercial scale.

Conditions for normal cooking of legumes soaked in water alone by were conducted by past investigators. Pastuszewska et al. [95] has reported, 1 h to cook after soaking 18 h in room temperature for peas. Nergiz and Gokoz [5] has reported, 40 min to cook after soaking 12 h at room temperature for dry beans. Mubarak [98] has reported, 90 min to cook after soaking 12 h at room temperature for mung beans.

Conditions for pressure cooking of legumes with soaked in water alone were conducted by past investigators: Mubarak [98] has reported 35 min to cook by autoclaving at 15 lb atmospheric pressure, (121°C) after soaking 12 h in tap water (1:10, w/v) for mung bean seeds. Nergiz and Gokoz [5] has reported 40

min to cook without soaking and adding the distilled water (1:5 w/v) for dry beans.

Results for microwave cooking was noted as 15 min to soften 50% of the seeds felt between fingers, after soaking for 12 h with tap water (1:10, w/v) for mung bean seeds [98].

3.6.1 The factors affecting the cookability of legumes

Cooking ability of legumes affects the eating quality. Therefore it is important to understand the basis of the changes in hardness of the cooked bean. This would allow process parameters to be optimized for better eating quality.

Storage conditions of high temperature and relative humidity are critical constrains to consumption of legumes and it has been reviewed that elevated moisture content and temperature reduce cook-ability for many legumes. This phenomenon is called as the "Hard to cook' (HTC) defect related to impermeability of the seed coat to water and it is observed in many different legume genera and species [32]. The cooking time of legumes depends primarily on the softness of the cooked seeds and it may vary depending on the type of legume. The large variations in seed characteristics among the different types of legumes also have direct influences on the soaking and cooking characteristics of seeds [100].

It has been reviewed that, during storage Ca^{++} is released from calcium phytate complexes and migrates to the cell middle lamella where it binds to the carboxyl groups of pectin, insolubilizing it, forming a barrier to water penetration and cell separation during cooking and was reported as a cause for hardness of seeds even after cooking. Some investigators have hypothesized that lignifications through cross-linking of phenolic compounds in the cell walls produces similar results confirmed that storage at elevated temperature and relative humidity caused the firmness of cooked legumes to increase and showed that the degree of hardening was a linear function of time and non-linear function of temperature and relative humidity [32]. The effects of processing vary notably, depending on the techniques and conditions, including time, temperature, moisture content and pH [102]. Some authors have noted that brown legumes which were stored for different periods of time had varying nutritional value after cooking. Freshly harvested beans rapidly imbibed water on soaking for 16 h, while of those at one-year-old, 25% did not imbibe water during the same soak period.

Extreme storage conditions may result in seeds that are simply impossible to cook, and that the high mineral (multivalent cat-ion) content of soaking/cooking water may further reduce cook ability [32].

Legumes, stored under various conditions have not been fully investigated, especially textural properties [54].

3.6.2 Effect of combination of soaking and cooking methods

Cooking has different effects on nutritional and anti nutritional compounds of legumes depend on species and cooking time.

It has been noted that there is a very significant loss of dry material during soaking and cooking. Although there is evidence that some of these losses are desirable (oligosaccharides of the raffinose family), it is also true that the cooking liquid is also often used to prepare dishes. From the nutritional viewpoint, it would be highly recommendable to skip the soaking stage or to retain the same water for soaking and cooking [6]. The losses in protein could be attributed to partial removal of certain amino acids along with other nitrogenous compounds on heating, as has been reported [103]. There was no significant difference between the protein contents of pressure- and microwave-cooked legumes for Bengal gram, green gram and horse gram [104]. A slight decrease in the crude proteins was observed in the cooked peas. The raw seeds contained 27.2% of CP, while the CP content of cooked peas varied from 25.6 to 26.3% (dry weight basis) according to the time and method of cooking. This decrease was probably due to leaching of water soluble proteins into cooking water [102]. Cooking whole dry seeds of chickpea caused significant decreases in protein [105]. Boiling and microwave cooking caused a slight increase in total essential amino acids, but the value was not influenced by autoclaving. Cooking treatments decreased the concentration of lysine (except microwave cooking), tryptophan total aromatic and sulfur amino acids [91]. He has further reviewed that, cooked and germinated chickpea seeds were still higher in lysine, isoleucine (except autoclaving) and total aromatic amino acid contents than the FAO/WHO reference pattern and these results were reported by previous investigators who found that cooking reduced sulfur containing amino acids and tryptophan and reviewed [91].

The amount of water associated with the protein can markedly affect the thermal stability and potential application of food proteins. The temperature and heat-moisture conditions are of great importance [106]. Therefore, losses of nutrients during normal cooking can be controlled by the amount of cooking water and its drainage [105]. The combined effect of soaking and water blanching on nutrient losses were greater than that of soaking and steam-blanching [107].

Extrusion of legumes a priori soaked in water for 16 h is recommended to improve the nutritive value of these legumes in order to increase its utilization in human and animal nutrition either consumed directly or as an ingredient of certain meals [96].

When considering vitamins, it has been investigated that riboflavin, thiamin, niacin and pyridoxine in chickpea seeds were significantly reduced by cooking treatments. These losses were probably due to a combination of leaching and chemical destruction. The losses by microwave cooking were smaller than those

obtained with boiling and autoclaving. The improvement in vitamin retention by microwave cooking may have been due to the shorter cooking time compared to boiling and autoclaving. The sensitivity of vitamins to loss from cooking was in descending order: pyridoxine, riboflavin, thiamin and niacin. Boiling resulted in a greater loss for each vitamin compared to the other cooking treatments. Conventional cooking caused a high loss of thiamin, riboflavin and ascorbic acid in vegetables, but microwave cooking and autoclaving improved the retention of these vitamins compared to boiling [91].

Since most of the anti-nutritional compounds such as trypsin and chymotrypsin inhibitors, lectins, tannins, phytic acids and amylase inhibitors are heat-labile, there are variations in the elimination of anti-nutrients depending on the method of processing. It has been reported that cooking has different effects on legumes since it removes TIA, increases the level of compounds determined as dietary fibre, and considerably reduces the tannin content in coloured flower peas and effect on its in vitro protein digestibility [95].

As noted under topic 1.3 and Table 2 the remedies to overcome anti-nutrients, past authors have investigated that anti-nutrients in legumes can be eliminated by different methods of cooking. Combined soaking and boiling is an effective method of reducing most of anti nutritional factors [35]. Further, the highest reduction of Trypsin Inhibitor activity was noted after autoclaving (83.67%) followed by boiling (82.27%), microwave cooking (80.50%) and germination (33.95%). It has been reported that trypsin inhibitor activity of soybeans decreased by about 12% after 12 days of germination. Hemaglutinin activity was completely destroyed by cooking and was drastically reduced (77%) by germination [91]. In commercial scale, extrusion cooking at temperatures ranging from 100-148°C was found the best way: to remove anti-nutritional factors in legumes, for processing such that to permit consumption without any health risk factors and for well being not only for human but also for animal feed as well.

Cooking significantly improved the protein digestibility (9.9-11.8%) and considerably reduced the TIA (53.6-59.9%), the phytic acid (24.0-34.5%) and the polyphenol contents (58.7-62.6%) [105]. Combination of soaking and water-blanching and soaking and steam-blanching had significant effects on the reduction of TIA and oligosaccharides when compared with single processes [107]. A reduction in the level of anti-nutrients from the different food legumes was also observed as a result of the different hydrothermal treatments [103].

3.7 Digestibility of legume proteins

Protein digestibility is a primary determinant of the availability of amino acids. Therefore, protein digestibility is important in evaluating the nutritive quality of a food protein [61]. The low content of essential sulfur amino acids,

the compact structure of the major proteins and the presence of anti-physiological proteins, protease inhibitors and non-protein compounds have been deemed [108]. It has been reported that albumins were much less digestible than globulins, mainly because of the primary structure, native conformation, involvement of disulphide bonds in the formation of complexes and its poorly susceptible to proteolysis. The low digestibility of globulins has been related to their compact structure and intracellular location that hinder the susceptibility to proteolysis [109]. Apart from these, methods of processing also affect on digestibility of legume proteins.

A number of papers in the literature on the nutritional value of legume proteins suggest that plant proteins are less susceptible to proteolytic breakdown in vivo digestibility than animal proteins.

With respect to soaking of legumes, the increase in IVPD after soaking may be related to solubility of protein because of water imbibitions. Improvement of IVPD was noticed when dhal was soaked for different durations. Protein digestibility increased considerably up to 24 h soaking in case of chick pea, whereas the increasing trend was noticed up to 36 h soaking in case of pigeon pea [14]. According to a study of IVPD of raw un-soaked kidney beans showed the lowest IVPD (70.59%), while the soaked legumes had comparable values (74.0-75.4%) [94].

With respect to cooking, the IVPD of raw vegetable peas was 73.5% and was improved by cooking. The highest IVPD (78.3%) was obtained by pressure cooking (15 min) or by ordinary cooking (40 min), whilst the least improvement was noticed in the microwave-cooked vegetable peas (4 min) [102]. Cooking significantly improved the protein digestibility (9.9-11.8%) [105]. When dry beans subjected to soaked-cooking processing had 81.27% digestibility on an average. An improvement (14.88%) occurred in the digestibility in comparison with the raw samples [5].

Pressure-cooked samples additionally had a value of 79.2% in the protein digestibility on an average. This method caused less improvement (11.9%) than the soaked-cooking method in digestibility. The findings are in good agreement with the reported values by Abd El-Hady and Habiba [96]. Improvements in dry bean protein digestibility might be attributed to the removal of anti-nutrients and the inactivation of enzyme inhibitors by heating [5]. Maximum improvement in protein digestibility (68.0-76.0%) was observed on cooking black grams, chickpeas, lentils, red and white kidney beans in an autoclave at 121^{o}C for 10 min. However, a gradual decline in protein digestibility was observed, whereas starch digestibility remained unchanged as the cooking time increased from 10 to 90 min and the temperature increased from 121 to 128°C [103].

Extrusion processing enhanced the IVPD in all studied legumes. For example, the IVPD of the faba beans increased from 75.4% in raw un-soaked seeds to 80.4% in soaked extruded seeds at 140°C and 18% moisture content. Further,

the kidney beans IVPD increased from 70.59% in un-soaked raw seeds to 79.26% in soaked extruded seeds for the same condition. These results agree with those of Alonso et al. [38] and in extruded peas ABD El-Hady and Habiba, [96].

When consider germination, a steady increase in IVPD of cow pea (*Vigna unguiculata*), red gram (*Cajanus cajan*) and green gram (*Phaseolus aureus*) were noted as the germination progresses [14]. Further, it was reported that high-PD in the initial stages of germination. There are indications that germination is effective increasing protein digestibility and improving sensory properties due to reducing phytic acid and flatulence caused by oligosaccharides (namely stachyose and raffinose). In case of white kidney beans, faba beans and chickpeas; sprouting improved the protein/amino acid digestibility by decreasing anti-nutritional factors and increasing the true/apparent protein/ amino acid digestibility [11].

All cooking treatments and germination improved the IVPD and protein efficiency ratio of chickpeas. Even though, germination improved the IVPD of mung beans, it did not find any effect of germination on IVPD of cowpeas. The improvement in digestibility may be attributed to de-naturation of protein, destruction of the trypsin inhibitor or reduction of tannins and phytic acid [91]. Chrenkova et al. [19] have reported that extruded peas provide the best growth performance without negative effect on carcass characteristics and meat quality of growing finishing pigs.

Though many studies have been carried out in legumes, there is very limited information on the legumes under study especially with regard to protein quality by different methods of processing.

4. MATERIALS AND METHODS

4.1 Design of experiment

According to the scope of this thesis, to find the ways to utilize legumes effectively, this research was planned to achieve the set targets and goals as given in Figure 1 and the main components of experiment design that carried out for legume seeds are shown in the figure below.

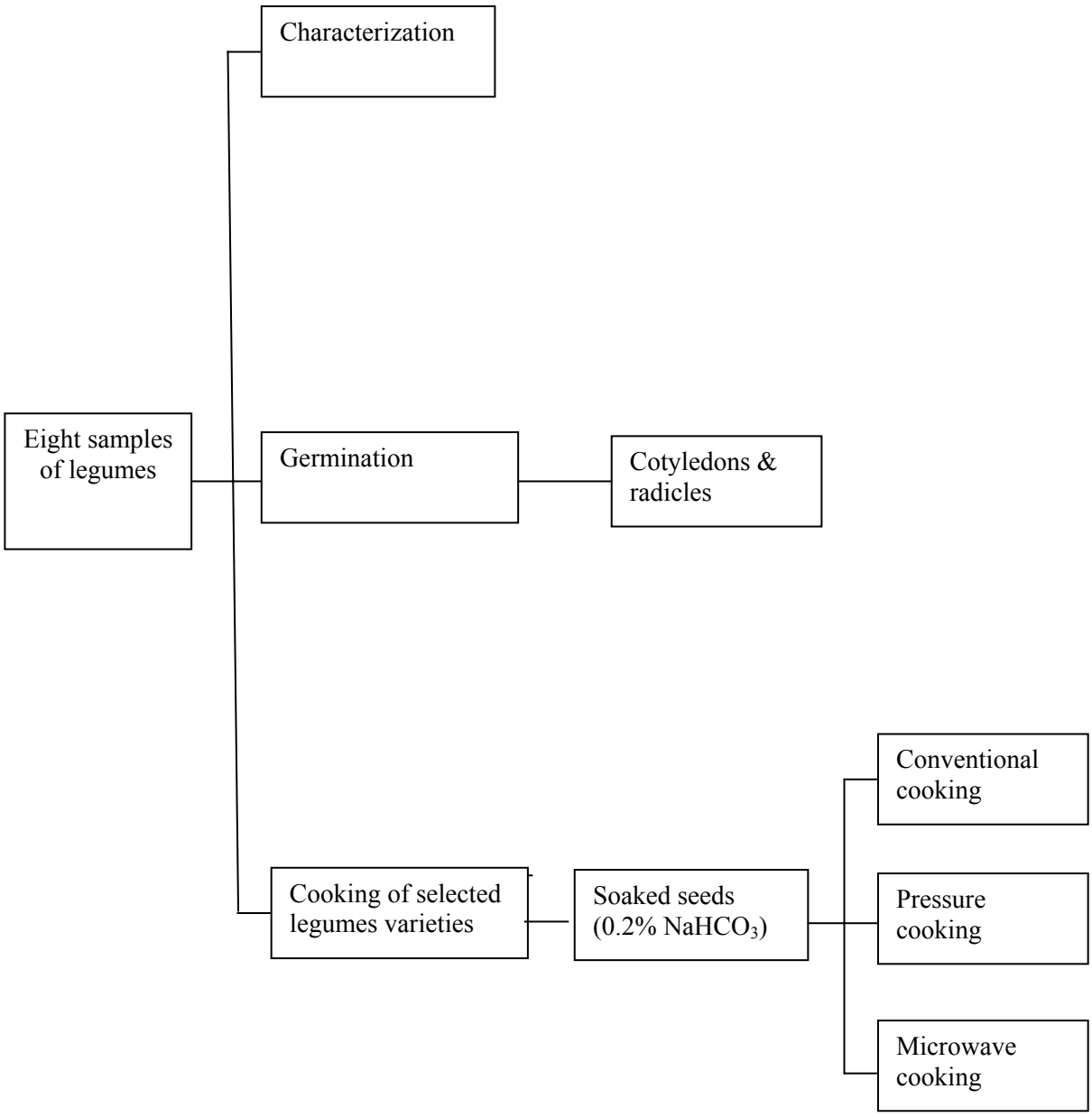

Figure 3. Main Components of experimental design

Eight legumes samples used for this study were obtained from the Food Research Institute in Slovakia. *Pisum sativum* (cultivars: Xantos, Achat, Svit, Terno), *Pisum sativum* var. *arvense* (Arkta), *Lupinus albus* (Amiga), *Glycine max*, *Faba vulgaris (*Piešťanský) shown in Figure 5, were employed for all determinations. The seeds were stored in the containers at room temperature until used.

Though many studies have been carried out on legumes, there is lack of detail information regarding the legumes considered in this study. Accordingly, as shown in the Figure 3, in stage I, all legumes were analysed for dry matter, ash, Crude fat, crude fiber, crude protein, amino acid, digestibility of protein, dry matter, organic matter and fractionation of proteins for characterization of legumes.

Further, with regard to germination of legumes, though many studies have been carried out, there is scarcity of information regarding the protein quality of cotyledon and radicle separately after germinating. Since many researchers have reported that digestibility of proteins and availability of amino acids major factors to determine their protein quality assessment [61], it is imperative to evaluate protein quality of cotyledons and radicles with aim of assessing the nutritional value.

Based on past evidence since the long period of germination has negative effect on sensory quality, in stage II, all legumes were germinated for 48 h and amino acids, crude protein, *in vitro* digestibility of dry matter and protein were evaluated for cotyledons and radicles and it was compared with raw seeds.

In domestic scale legumes consume only after cooking. There is very limited study with regard to digestibility of legumes considered in this study with different methods of cooking and with different cooking times.

Therefore, in stage III only two cultivars of *P. sativum* (Xantos and Svit) and *G. max* were used for cooking. Legumes in each selected legume cultivars were soaked in 0.2% $NaHCO_3$ solution (seeds: soaking solution; 1:5 w/v) for 6 h at room temperature (25°C) and were cooked by conventional cooking, pressure cooking and micro wave cooking. The samples were collected in four different time cooking times in each of the cooking methods for analyzing nutritional value.

The changes of nutritional value of selected legumes for cooking were compared with the same legumes that were germinated in stage II and with raw seeds in stage.

4.2 Description of legume samples

Description of the legumes samples given below were obtained from the Food Research Institute in Slovakia.

Pisum sativum (Xantos)

Yellow seed semi leaflet variety, with reduce leaf plane, intermediate medium growth, use for production of dry seed and forage purpose, high crop of seed, weight of 1000 seeds is 259g, average content of protein, but its crop is higher, middle content of inhibitor trypsin.

Technologic feasibility: seed soaking, boiling and steadiness of seed cooking are good.

Pisum sativum (Achat)

Green seed semi leaflet variety, with reduce leaf plane, use for production of dry seed for human and animal nutrition, resistant for radicular illness, ilness ascochyta, perenospora and mosaic disease is good. Weight of 1000 seeds is 259 g. For maintenance of green seeds colour is important, harvest when 18% of moisture and dry. Average content of protein and content of trypsin is below the average.

Technologic feasibility: seed soaking, boiling and steadiness of seed cooking are good.

Pisum sativum (Svit)

Yellow seed and semi leaflet variety with resistant for *fusaria* and *Erysiphe pisi.* Seed contain average content of protein and trypsin inhibitor.

Pisum sativum (Terno)

Medium late yellow seed semi leaflet variety, with reduce leaf plane, higher intermediate growth, used for production of dry seed for food purpose, high crop, resistant for radicular and cervix illness

Seed is great with high content of protein.

Faba vulgaris (Piestansky)

Medium early variety, great food quality, good seeds boiling, beige colour seeds, harvest of seeds (2.5-3.5 cm, oblate, wedge – shaped form) in milk –line maturity.

Lupinus albus (Amiga)

White sweet lupin has no alkaloid, medium early variety, middle high plants about 64.0 cm. Weight of 1000 seeds is ranged 300 - 350 g, blue colour of flowers, white seeds without bitter substance, good take in of animals, reward of soya been, above- average crops, content of protein is ranged 31-34%, content of fat 11.5%.

Pisum sativum var. *arvense* (Arkta)

This field pea is a winter variety, fast spring growth, high crop. High amount of crude protein, less weight of 1000 seeds, crop of seed 73%, used for green feed or silage.

Glycine max

Legume native to East Asia, cultivation is successful in climates with hot summers, yellow seed, rich source of protein up to 40%, use for processed in a variety of ways, approximately 60% of production used in animal feed. Other major uses include cooking oils, margarine, tofu and other inputs into human diets.

Figure 5. Samples of all legumes used for the analysis

4.3 Methods of processing and sample preparation

4.3.1 Raw seeds samples

Seeds were ground by using a household flourmill (Braun, Germany) and samples were preserved in air-tight bottles in the refrigerator for analysis.

4.3.2 Germinated seeds samples

Method of Khalil et al. [110] was followed with a few modifications. Seeds were sterilized by using 70% ethanol for 1 minutes, were soaked in distilled water for 16 h, kept on sterilized petri dishes, lined with filter paper and allowed to germinate in the dark for 48 h. Sprouts were washed and sprayed with distilled water twice for every 24 h during germination periods at 6 a.m. and 6 p.m. and used as fresh form for further analysis.

4.3.3 Cooked seeds samples

Soaking: Method of Vijayakumari et al. [13] and Kadam et al. [99] was followed with a few modifications in $NaHCO_3$ concentrations. Legumes were soaked in 0.2% $NaHCO_3$ (1:5, w/v) for 6 h at room temperature (25°C). The soaked seeds were drained and rinsed three times with distilled water, then cooked by the methods described below:

Normal cooking: Methods of past workers were followed with a few modifications in cooking times. Rinsed soaked seeds are cooked in tap water (100°C) in the ratio of 1:4 (w/v) on a hot plate and samples were collected in four time intervals (20, 25, 30, 35 min) [95,98].

Pressure cooking: Methods of past workers were followed with a few modifications in cooking times. Soaked seeds were pressure cooked in house hold pressure cooker at 103.42 Kpa (15 lb pressure), 121°C in tap water (1:4, w/v) and samples were collected in four time intervals (8, 10, 12, 14 min) [98].

Microwave cooking: Methods of past workers were followed with a few modifications in cooking times. Rinsed soaked seeds were placed in a pirex pot with tap water (1:4, w/v), then cooked in a microwave oven (Goldstar, Model ER-50540, 2450 MHz) and samples were collected in four time intervals for 8, 10, 12, 14 min [98].

All cooked samples were kept at –80°C in freezer and lyophilized at –40°C, 0.12 milli bars for 48 h (ALPHA 1-4 LSC). Then samples were ground and flour packaged in heat sealed vacuum bags and refrigerated at 4°C for the further analysis [98].

4.4 Methods of analysis

Chemicals and reagents:

All chemicals and reagents were either from Sigma chemical company (St. Louis, MO) or of analytical grade.

4.4.1 Basic chemical analysis

Dry matter of ground samples were dried in an oven at 105±1°C to constant weight for 3 h dry matter (DM) the standard procedure of AOAC [111].

Grounded legume samples and germinated legume were dried in an oven at 105±1°C for 24 h and the percentage (% w/w) of final weight is expressed as dry matter content for anlysing IVPD and dry matter digestibility.

Grounded legume samples were kept in muffle furnace at 550±5°C for 6 h and the percentage (% w/w) of final weight was expressed as ash content according to the methods of AOAC [111].

4.4.2 Crude protein

Extraction of Nitrogen by Mineralization

Chemicals: H_2SO_4 (98% w/w), H_2O_2 (30% w/w), catalyst $CuSO_4$.

Mineralization was carried out by the methods of by using mineralization unit to extract N to determine crude protein.

Crude protein (CP) content was determined by the micro-Kjeldahl method after mineralization by mineralization unit. CP was determined by micro-Kjeldhal method using a nitrogen autoanalyser (Auto-titration Kjeldahl distiller, Pro-Nitro 1430) and nitrogen to protein conversion factor of 6.25 was used [111,112]

Chemicals used

H_2SO_4 (98% w/w), HCl (0.102 mol.l-1, H_2O_2 (30% w/w), H_3BO_3(2% w/w), 30% NaOH, Tashiro's Indicator

4.4.3 Crude fat

Chemicals used- Petroleum ether

Soxhlet extraction

Three gram of dried legume seed sample was weighed in to an extraction thimble. The flask use for fat extraction was weighed accurately. Thimble was put in to a Soxhlet extractor and the flask was connected. Sufficient amount of petrolium ether was poured in to extractor. The extractor was connected to the water condenser and the flask was heated on water bath about 6-8 h until

extraction complete. Then flask was removed and placed in oven at 100°C to evaporate the last traces of solvent and to dry for a constant weight [111,113].

4.4.4 Crude fiber analysis

Sample were hydrolyzed in filter bags (F 57, pore internal dimension 50 μm, (ANKOM 200/200 Fiber Analyzer, New York) by using 127.5 mM H_2SO_4 and 313 mM NaOH separately, 45 min. Then, filter bags containing samples were washed in water (three times) and dipped in acetone for 3 min and allowed to dry. After acetone evaporation, the bags were dried at 105±1°C (4 h) and than incinerated in muffle furnace at 550°C (5 h) [114,115].

The Crude fiber (CF) were calculated according equation below:

$$CF = \frac{(m_3 - m_1 c_1) - (m_4 - m_1 c_2)}{m_2} \cdot 100$$

where *CF* is content of crude fiber in %

m_1: weight of bag (g)

m_2: weight of legume seed sample (g): 1 g (for CF)

m_3: weight of dried bag with hydrolyzed sample (g)

m_4: weight of bag with hydrolyzed sample after incineration

Corrections c_1 and c_2 were calculated according following equations:

c_1 is correction of bag weight after hydrolysis

c_2 is correction of bag weight after incineration

$c_1 = m_S/m_1$

$c_2 = m_P/m_1$

(m_s…weight of dried bag after hydrolysis, m_P…weight of bag ash)

4.4.5 Amino acid analysis in legumes

All grounded legumes samples are subjected to acid hydrolysis with 6 M HCl at 110°C for 23 h [75,76,77,78]. Amino acids containing sulphur is hydrolysed separately with 6 M HCl after oxidizing (formic acid + hydrogen peroxide, 9:1 v/v, 20 h at 4±1°C). Amino acids were determined by using an AAA 400 amino acid analyser (INGOS, Czech Republic) with ion exchange chromatography [75] with post column (column 370x3.7mm) ninhydrin-based detection by using Sodium citrate buffer. The ninhydrin amino acid derivatives were detected at 570 nm for primary amino acids and detection at 440 nm for secondary amino

acids. Amino acid contents were calculated by using retention time of chromatograms (shown in Appendix only one sample of legumes as an example) as given in Appendix and according to the manual [116,117].

4.4.6 *In vitro* digestibility of protein and dry matter with pepsin

The *in vitro* digestibility of ground seed legumes were determined by using pepsin (3 g/1.5l of 0.1M HCl in to one jar; pepsin EC 3.4.23.1. from porcine gastric mucosa, Merck, Darmstadt, Germany). Sample size of 0.25-0.40 g weigh in to each bag, heat sealed and place in the Daisy^{II} Incubator digestion jar (up to 25 samples per jar). Digestion is set at 39±1°C 24 h [101,115]. Then the Jars kept at 80±1°C for 30 min to dissolve starch and bags are washed thrice with distilled water and kept in the oven at 105±1°C for 24 h and record the dry weight and undigested protein is measured by the micro Kjeldahl method and calculated as IVPD. Same method is carried out to analyze the digestibility of organic matter by keeping all bags in muffle furnace and record the weight of ash after drying [109].

Values of digestibility of dry matter (D_{DM}) and organic matter (D_{OM}) were calculated according following equations:

$$DMD = 100 - \frac{100 \cdot DMR}{m_2 \cdot DM}$$

$$DMR = m_3 - m_1 c_1$$

$$DM = \frac{DM_{\%} \cdot m_S}{100}$$

$$OMD = 100 - \frac{100 \cdot (DMR - AR)}{m_2 \cdot DM \cdot OM}$$

$$AR = m_4 - m_1 c_2$$

$$OM = \frac{DM_{\%} - A_{\%}}{100}$$

DMD: digestibility of legume dry material in %
OMD: digestibility of organic matter in %

DMR: weight of sample after incubation and drying (g)

DM: dry matter (g)

$DM_{\%}$: dry matter (%)

AR: weight of ash of sample (g)

OM: organic matter in dry matter (g);

$A_{\%}$: ash content after sample incineration (%)

m_1: weight of bag (g); m_2: weight of sample (g)

m_3: weight of dried bag after incubation (g)

m_4: weight of ash of bag and sample after incubation (g)

m_S: weight of sample for dry matter determination (g)

Corrections c_1 and c_2 were calculated according following equations:

c_1 is correction of bag weight after incubation

c_2 is correction of bag weight after incineration

$c_1 = m_S/m_1$

$c_2 = m_P/m_1$

(m_s…weight of dried bag after incubation, m_P…weight of bag ash)

4.4.7 Isolation and fractionation of proteins

Ground legume seeds were weighed out in to 50 ml centrifugal tube and solutions were added for fractionation.

Twenty five ml of distilled water was added to each centrifugal tube and allowed shaking 1hour in an electric shaker for 1 h. Then it was allowed to centrifuge 10 min at 4000 rpm temperature of 20±1°C (HERMLE z300k, Germany). Supernatant was separated in to 50 ml volumetric flask and residue was re-extracted with 25 ml of the same solvent and recovered supernatants were combined and make up to 50 ml. It is designated as albumin.

The residue was then extracted successively with 5% NaCl, 70% (v/v) ethanol at 65±1°C for 1 h a shaking water bath, and 0.1 M sodium hydroxide to separate the total seed proteins into albumin, globulin, prolamine and glutelin fractions, respectively. Residue was allowed to dry at 105±1°C for 3 h and store until use for protein analysis [61].

CP of fractionated samples were determined by using 5 ml of protein solutions and calculated each of protein fraction content (%) as in equation 1 for each of the fractions [111].

4.5 Statistical Analysis

All determinations were in 10 replicates and standard deviations (SD) were calculated. Data were evaluated by producing summary statistics and analyzing the variance using an ANOVA. The mean values are separated by using Dunken multiple range test and Wilcoxan signed rank test to determine significant differences ($P < 0.05$) [118,119].

5. RESULTS AND DISCUSSION

5.1 Characterization of legumes

5.1.1 Basic chemical composition of raw legumes

The summary of the basic chemical composition of some legumes is presented in Table 3 and 4. The DM content of all legume seeds were noted as uniform and it was above 90% and the highest amount was noted in *G. max* (93.5%). The ash content of the whole seeds ranged from 2.7-5.3% while the lowest was in *P. sativum* (Xantos) and the highest was in *G. max*. Ash content is significant in food for various reasons. It is an index for of the quality of feeding materials used for animals. Therefore, the studied legumes could be used for animal feed as well.

The CP contents of the seeds are fairly high and it ranged from 21.5-34.4% which is in agreement with past investigations [4,20]. *G. max* had the highest value CP and *L. albus* also had the more or less similar value to *G. max* while the lowest was in *P. sativum* var *arvense*. Among the cultivars of *P. sativum* in the present study revealed that CP values of *P. sativum* (Terno) is higher than those of other legumes. In some cases, CP value is higher than those recorded for similar seeds by past investigators. According to Costa et al. [98] protein values of the legumes ranged from 18.5 to 21.9 g/100 g for pea, lentil and beans and from 21.3 to 23.7 g/100 g for freeze-dried cooked legumes. The crude fat content of most of legumes tested was low and in the range between 1.1- 1.4% except *G. max* and *L. albus*. *G. max* had the highest content of crude fat 18.7% is followed by *L. albus* having 7.4%. According to Costa et al. (2006) [98] pea lipid ranged from 2.3-2.6%.

The crude fibre content ranged from 5.7 to 7.6% in all the legumes tested and with exception of *L. albus* having 16.2% being the highest. The crude fiber content for *P. sativum* are some what lower to the result of Costa et al. [97].

The lowest NFE was noted in *G. max*, followed by *L. albus* which were 27.6% and 30.1% respectively and in other legumes it ranged from 50.8 to 59.9%. There is not much variation in organic matter and it ranged from 86.1-88.6%.

The results of *P. sativum* are comparable to the pea cultivars studied by Pastuszewska et al. [95]. It did not differ appreciably from those previously reported for pea varieties by past investigators [75,120,121]. Results of *L. albus* were also in agreement with Makri et al. [121].

From a nutritional point of view, the studied legumes had high nutritional composition and are supportive of the utilization of peas (*P. sativum*), faba beans (*F. vulgaris*) and lupin seeds (*L. albus*) as acceptable substitutes for soybean as well as an effective protein source.

Table 3. Proximate composition of raw seeds in % w/w of some varieties of *P. sativum*

Parameter tested	*Pisum sativum*			
	Terno	Xantos	Svit	Achat
Dry matter	90.68 ± 0.019	91.52 ± 0.087	91.36 ± 0.091	91.52 ± 0.104
Ash	2.80 ± 0.051	2.74 ± 0.068	3.03 ± 0.109	2.93 ± 0.093
Crude protein in dry matter	24.21 ± 0.218	21.98 ± 0.179	23.16 ± 0.358	22.44 ± 0.508
Crude fat in dry matter	1.13 ± 0.059	1.20 ± 0.107	1.09 ± 0.087	1.14 ± 0.073
Crude fiber in dry matter	7.64 ± 0.207	5.75 ± 0.193	5.91 ± 0.093	6.15 ± 0.106
Organic matter	87.88 ± 0.129	88.78 ± 0.106	88.33 ± 0.122	88.59 ± 0.113
Nitrogen-free extract	54.90 ± 0.103	59.85 ± 0.141	58.17 ± 0.109	58.86 ± 0.117

Data shown are Mean ± SD; n =10

Table 4. Proximate composition of raw seeds in % w/w of some varieties of *G. max, L. albus, P. sativum* var. *arvense* and *F. vulgaris*

Parameter tested	*Glycine max*	*Lupinus albus*	*Pisum sativum* var. *arvense*	*Faba vulgaris*
		Amiga	Arkta	Piestansky
Dry matter	93.52 ± 0.111	92.13 ± 0.079	90.43 ± 0.091	91.72 ± 0.168
Ash	5.33 ± 0.049	4.42 ± 0.198	4.31 ± 0.251	3.49 ± 0.139
Crude protein in dry matter	34.39 ± 0.401	33.97 ± 0.528	21.53 ± 0.395	29.26 ± 1.581
Crude fat in dry matter	18.69 ± 0.047	7.37 ± 0.106	1.40 ± 0.079	1.26 ± 0.039
Crude fiber in dry matter	7.54 ± 0.133	16.24 ± 0.161	7.62 ± 0.105	6.94 ± 0.201
Organic matter	88.19 ± 0.219	87.71 ± 0.173	86.12 ± 0.166	88.23 ± 0.234
Nitrogen-free extract	27.57 ± 0.139	30.13 ± 0.201	55.57 ± 0.151	50.77 ± 0.137

Data shown are Mean ± SD; n =10

5.1.2 Amino acid composition of raw seeds of legumes

The results of the amino acid profiles in g/16g N of the studied legume cultivars in Table 5 and 6, revealed that the proteins of this seeds contained adequate levels of essential amino acid (EAA) comparable with the FAO/WHO [58] except for the phenylalanine in all legumes. Further, the results are comparable with *P. sativum* by Iqbal et al. [75] and *G. max* by Vasconcelos et al. [52], *L. albus* by Sujak et al. [56].

Although sulphur containing amino acid had been reported to be limiting amino acids for many legumes [81,89], a higher level of methionine was found in raw seeds of *P. sativum* (Terno), *Glycine max, L. albus* (Amiga) and *P. sativum* var. *arvense* (Arkta) which is 5.0, 5.5, 5.2, 5.4 g/16gN respectively when compared with other legumes tested and requirements pattern of FAO/WHO [58]. This may be due to changes of nutrients in the soil where they are grown, climatic and environmental conditions [4]. As reported by past authors, it is often desired to have plant seed proteins that have higher levels of sulfur-containing amino acids [65] and therefore, these legumes can use to meet nutritional requirements.

Significant variation existed in the EAA, particularly the highest amount of arginine and the contents were varied from 11.7g/16g N in *F. vulgaris* to 7.7g/16gN *in G. max* and all *P. sativum* varieties had higher amount of arginine than in Pakistan variety tested by Iqbal et al. [75]. All legumes tested were found to be rich in lysine, leucine and arginine. This is in agreement with Iqbal et al. [75]. Glutamic acid and aspartic acid were found to be major the NEAA in the sample tested. Relatively low concentrations of cystine were observed in all which ranged from 1.7g/16gN in *P. sativum* (Achat) and 2.5g/16gN in *L. albus*. Soya bean has reported as one of the most important legumes from the standpoint of nutritional value [82]. However, the highest TEAA found in *P. sativum* var. *arvense* (48.2g/16gN) while the lowest of TEAA (42.4g/16N) and the highest NEAA in *F. vulgaris*. Most of the amino acids were more or less similar in both *G. max* and *L. albus* and found to be higher when compared with the other legumes except *P. sativum* (Terno) and *P. sativum* var. *arvense.* The major amino acids found in all eight species were aspartic acid, alanine, and glutamic acids. The highest and the most prominent amino acids found in the above tested legumes was aspartic acid, glutamic acids and arginin.

Figure 3. shows the variation on TEAA, NEAA, TAA contents in g/16gN. TAA is highest (97.4%) in *L. albus* while the lowest (88.3%) in *P. sativum* (Achat). The highest TEAA (47.9g/16gN) was in *P. sativum* (Terno) among the *P. sativum* tested. Among the *P. sativum* cultivars, methionin content was highest in *P. sativum* (Terno) i. e. 5g/16gN. *P. sativum* var. *arvense* has the highest TEAA (48.2g/16gN) among all legumes tested.

Table 5. Comparision of amino acid composition of some varieties of *P. sativum* grown in central Europe with Asian and FAO reference pattern (g/16g of N)

Amino acid	*Pisum sativum*				Green Pea**	FAO/WHO 1991 *
	Terno	Xantos	Svit	Achat		
Arg	9.4 ± 0.22	8.6 ± 0.45	9.7 ± 0.22	8.3 ± 0.45	7.2 ± 0.04	
His	2.2 ± 0.12	2.2 ± 0.11	2.2 ± 0.01	2.2 ± 0.08	2.4 ± 0.05	1.9
Ile	4.2 ± 0.09	3.9 ± 0.20	3.8 ± 0.02	3.9 ± 0.09	4.5 ± 0.06	2.8
Leu	7.1 ± 0.16	6.5 ± 0.29	6.3 ± 0.07	6.6 ± 0.21	7.4 ± 0.05	6.6
Lys	6.9 ± 0.20	6.6 ± 0.26	6.4 ± 0.03	6.6 ± 0.21	8.1 ± 0.07	5.8
Met	5.0 ± 0.06	1.1 ± 0.01	1.1 ± 0.06	1.0 ± 0.05	1.1 ± 0.03	2.5(+Cys)
Phe	4.9 ± 0.27	4.6 ± 0.20	4.3 ± 0.04	4.6 ± 0.13	5.2 ± 0.04	6.3
Thr	3.5 ± 0.03	3.6 ± 0.16	3.3 ± 0.14	3.5 ± 0.12	3.8 ± 0.05	3.4
Trp	n.a	n.a	n.a	n.a	0.8 ± 0.02	1.1
Val	4.7 ± 0.07	4.3 ± 0.19	4.3 ± 0.05	4.3 ± 0.17	5.0 ± 0.09	3.5
TEAA	47.9	41.4	41.4	41.0	45.5	33.9
Ala	4.2 ± 0.16	3.9 ± 0.17	3.8 ± 0.04	3.8 ± 0.24	5.2 ± 0.04	
Asp	10.9 ± 0.13	10.6 ± 0.34	10.7 ± 0.09	10.6 ± 0.43	11.0 ± 0.06	
Cys	2.0 ± 0.02	2.0 ± 0.06	1.9 ± 0.08	1.7 ± 0.14	1.8 ± 0.03	
Glu	15.1 ± 0.26	16.2 ± 0.67	16.0 ± 0.85	16.2 ± 0.68	17.5 ± 0.06	
Gly	4.1 ± 0.05	4.0 ± 0.14	4.0 ± 0.03	3.9 ± 0.21	4.5 ± 0.01	
Pro	3.8 ± 0.12	3.6 ± 0.15	3.6 ± 0.12	3.6 ± 0.14	3.8 ± 0.03	
Ser	4.3 ± 0.06	4.2 ± 0.18	4.2 ± 0.06	4.3 ± 0.22	5.1 ± 0.54	
Tyr	2.8 ± 0.10	3.2 ± 0.23	2.9 ± 0.11	3.2 ± 0.24	3.7 ± 0.03	
TNEAA	47.2	47.7	47.1	47.3	52.6	
TAA	95.1	89.1	88.5	88.3	98.1	

Data shown are Mean ± SD; n =10

*Data from FAO/WHO (1991) reference pattern of essential amino acid requirement for pre-school children (2–5 years old) [58].

** Iqbal *et al*. 2006 [75]

TEAA (Total essential amino acids), TNEAA (Total non essential amino acids), TAA (Total amino acids)

Table 6. Comparison of amino acid composition of some varieties of legumes grown in central Europe with *G. max* in other investigators (g/16g of N)

Amino acid	*Glycine max*	*Lupinus albus* Amiga	*P. sativum* var. *arvense* Arkta	*Faba vulgaris* Piestansky	*Glycine max* *
Arg	7.7 ± 0.33	11.0 ± 0.53	9.1 ± 0.69	11.7 ± 0.19	7.13
His	2.4 ± 0.13	2.3 ± 0.05	2.4 ± 0.13	2.4 ± 0.02	2.50
Ile	4.2 ± 0.17	4.3 ± 0.13	4.2 ± 0.23	3.7 ± 0.09	4.62
Leu	6.9 ± 0.25	7.3 ± 0.18	6.9 ± 0.36	6.5 ± 0.17	7.72
Lys	5.8 ± 0.30	4.7 ± 0.09	7.0 ± 0.29	5.6 ± 0.15	6.08
Met	5.5 ± 0.10	5.2 ± 0.02	5.4 ± 0.28	0.9 ± 0.06	1.22
Phe	4.7 ± 0.19	4.0 ± 0.08	4.8 ± 0.19	3.8 ± 0.11	4.84
Thr	3.6 ± 0.20	3.6 ± 0.12	3.6 ± 0.15	3.5 ± 0.25	3.76
Trp	n.a	n.a	n.a	n.a	n.a
Val	4.7 ± 0.23	4.3 ± 0.10	4.8 ± 0.23	4.3 ± 0.10	4.59
TEAA	45.5	46.7	48.2	42.4	42.46
Ala	4.0 ± 0.20	3.4 ± 0.29	4.2 ± 0.15	3.8 ± 0.10	4.23
Asp	10.3 ± 0.41	10.1 ± 0.22	10.7 ± 0.49	10.1 ± 0.27	11.30
Cys	2.4 ± 0.03	2.5 ± 0.02	2.4 ± 0.10	1.7 ± 0.08	1.70
Glu	15.6 ± 0.73	18.5 ± 0.60	15.2 ± 0.84	16.0 ± 0.95	16.90
Gly	4.3 ± 0.19	4.0 ± 0.15	4.4 ± 0.17	3.9 ± 0.09	4.01
Pro	4.6 ± 0.41	3.8 ± 0.19	3.9 ± 0.24	4.0 ± 0.22	4.86
Ser	4.3 ± 0.17	4.6 ± 0.11	4.3 ± 0.20	4.3 ± 0.22	5.67
Tyr	2.9 ± 0.11	3.8 ± 0.08	2.5 ± 0.18	3.1 ± 0.07	3.39
TNEAA	48.4	50.7	47.6	46.9	52.06
TAA	93.9	97.4	95.8	89.3	94.52

Data shown are Mean ± SD; n =10

* Vasconcelos *et al.* (1997) [52] and Siddhuraju & Becker (2005) [122]

TEAA (Total essential amino acids), TNEAA (Total none essential amino acids), TAA (Total amino acids)

Table 7. Percentage of protein fractions (%) in some legumes grown in Central Europe

Legume	Cultivar	Albumin	Globulin	Prolamin	Glutamin	Residue
Pisum sativum	Terno	44.1 ± 0.96	40.5 ± 0.09	4.5 ± 0.13	5.8 ± 0.01	4.3 ± 0.01
	Xantos	41.3 ± 1.50	42.0 ± 2.28	4.5 ± 0.07	6.2 ± 0.11	4.7 ± 0.21
	Svit	44.1 ± 0.48	40.3 ± 0.83	5.1 ± 0.01	5.1 ± 0.13	4.6 ± 0.01
	Achat	45.0 ± 0.35	41.6 ± 0.24	4.5 ± 0.11	4.1 ± 0.11	3.6 ± 0.06
Glycine max		48.5 ± 0.25	38.6 ± 0.65	3.6 ± 0.11	5.8 ± 0.07	2.8 ± 0.14
Lupinus albus	Amiga	40.3 ± 0.84	40.1 ± 1.23	5.3 ± 0.17	6.4 ± 0.10	7.2 ± 0.09
P. sativum var. *arvenes*	Arkta	45.9 ± 0.29	40.4 ± 0.58	4.3 ± 0.11	3.4 ± 0.09	5.4 ± 0.19
Faba vulgaris	Piestansky	45.3 ± 0.31	41.9 ± 0.36	3.5 ± 0.16	4.0 ± 0.14	3.6 ± 0.02

Data shown are Mean ± SD; n =10

5.1.3 Fractionation of protein in raw seeds of legumes

According to the results of protein fractionation presented in Table 7, albumin was found to be the main protein fraction in most of the legumes under study. This is in agreement with some cultivars of *P. sativum* studied by Martınez-Villaluenga et al. [123]. Globulin was slightly higher than the albumin fraction in *P. sativum* (Xantos) and *L. albus* (Amiga). Prolamine and glutelin were the minor protein fractions in all legumes. Similar results were observed for many legumes and recorded in past literature [8]. The ranges of the values obtained for albumins, globulins, prolamins and glutelins respectively in the legumes tested were 40.3-48.5%, 38.6-42.0%, 3.5-5.3% and 3.4-6.4% of the total extractable protein. The highest content of prolamin and glutelin were found in *L. albus* (Amiga), the respective values were 5.3 and 6.4%. Differences among cultivars were evident in all the legumes studied.

The highest level of albumin protein was found in *G. max*, followed by P. *sativum* var. *arvense*, *F. vulgaris* (Piestansky) , *P. sativum* (Achat, Terno, Svit, Xantos) and the lowest was in *L. albus* (Amiga) (Table 7). Information on protein fractions of these pea cultivars is not widely available.

During the separation of protein fractions co-precipitation may take place. This is more applicable in the separation of albumin and globulin, which are water soluble and salt soluble respectively. Therefore, the exact content of the respective proteins may not be extracted correctly and due to this reason, the measured values may result in an inaccuracy. The lowest content of globulin was observed in *G. max* being 38.6% and the content noted in other legumes, ranged from 40.1-42.0%. Although, inter- and intra species differences may exist in the albumin protein, globular protein, prolamin and glutelin content of legumes, these differences may be accentuated by the inaccuracies caused by the method of extraction as well [63].

5.2 Evaluation of nutritional quality of germinated of legumes

5.2.1 Amino acid composition of cotyledons and radicles of germinated legumes after 48 hours

Table 8 and 9 compiles the amino acid values of cotyledons and radicles after germinating 48 h in terms of 16 g/N. The tabulated results reveal that raw seeds displayed the higher proportions of amino acids when compared with cotyledons and radicles (after 48 h of germination) of the same variety with the exception of *P. sativum* (Xantos). It should be noted that EAA in *P. sativum* (Xantos) after germinating 48 h is in excess of the FAO/WHO [58] requirements while *G. max* and *F. vulgaris* (Piestansky), *P. sativum* (Achat) are comparable with most of EAA values recommended by FAO/WHO [58]. The decreasing of EAA with germination may be due to the amino acids produced by hydrolysis of the

protein reserves may also be used as an energy source, especially in the early stages of germination [10]. Further, certain amino acids more readily broken down than others, rearrangement, mobilization of the protein reserves in the cotyledons to synthesis of new proteins for sprout growth and increase in biogenic amines with germination time caused by enzymatic decarboxylation of amino acids [124]. Increase of non-protein amino acids may be caused due to alterations in the amino acids during germination [89]. This non-protein nitrogen substances consists of free amino acids, nucleic acids, puric and pyrimidinic bases, polyamines, alkaloids and small peptides [90]. King and Puwastien [125] have reported that amino acid composition for wing bean seeds decreases after 48 h of germination.

In this study, the highest TEAA of cotyledons (43.7%) and radicles (41.1%) was noted in *P. sativum* (Xantos) and in raw seeds (41.4%) of *P. sativum* var. *arvense* (Arkta) although soya bean has reported as one of the most important legumes from the standpoint of nutritional value [82].

Figure 7-15 show the variation of all amino acids in cotyledons and radicles after germinating 48 h vs. raw seeds of legumes. It was observed that high content of histidine, phenylalanine and alanine in the radicles of *P. sativum* (Xantos, Achat) and only histidin and alanin were found to be higher in radicles of *G. max*, when compared with their raw seeds and cotyledons.

The amount of alanin (NEAA) increases significantly in radicles of *P. sativum* (Xantos, Achat) and *G. max* when compared with the respective raw seeds and cotelydons. However, with respect to *L. albus, P. sativum* var. *arvense* (Arkta) and *F. vulgaris* (Piestansky) the amount of alanin in the radicles was comparable with raw seeds. Reduction of lysine with germination may be due to cadaverine, produced by enzymatic decarboxylation of lysine. It is concentrated mainly along the embryonic axis. Authors have suggested that it could play a role in sprouting and cell division [10,126]. Lysine, leucine and arginine were found to be the most prominent EAA in all cotyledons and radicles though they were some what lower than raw seeds while glutamic acid and aspartic acid were found to be the major NEAA in all cotyledons and radicles as in raw seeds. The EAA content of germinated seeds of *G. max* and *L. albus* cv. Multolupa of Brazil after 48 h of germination, is higher than the *G. max* and *L. albus* of Central Europe. However, methionine content is relatively lower of the selected legumes of Brazil, the values being 1.34g/16gN in *G. max* and 0.67g/16gN in *L. albus* cv. Multolupa [127].

Almost all amino acids have not changed in cotyledons and radicles after 48 h of germination in *F. vulgaris* (Piestansky) except arginine, glutamic and aspartic acid. Methionine and cystine were slightly increased in radicles of *P. sativum* (Xantos and Achat), *F. vulgaris* when compared to their raw seeds which may be due to synthesis. After bean germinating, histidine, glutamic, glycine, arginine, tyrosine contents were decreased, while aspartic, valine, isoleucine,

phenylalanine contents varied in different ways, depending on the germination conditions [1].

Table 8. Amino acid composition in cotyledons and radicles of some varieties of *P. sativum* after germinating 48 hours (g/16g N)

Amino acid	*Pisum sativum*							
	Terno		Xantos		Svit		Achat	
	cotyledons	radicles	cotyledons	radicles	cotyledons	radicles	cotyledons	radicles
Arg	7.7 ± 0.44	3.0 ± 0.24	8.5 ± 0.83	6.3 ± 0.63	6.7 ± 0.74	4.9 ± 0.00	8.2 ± 1.45	4.8 ± 0.54
His	1.6 ± 0.10	1.6 ± 0.13	2.2 ± 0.20	2.9 ± 0.24	1.4 ± 0.13	1.5 ± 0.11	2.0 ± 0.20	2.6 ± 0.19
Ile	3.3 ± 0.15	2.5 ± 0.12	4.2 ± 0.39	3.7 ± 0.22	2.7 ± 0.27	2.3 ± 0.03	3.9 ± 0.35	3.2 ± 0.22
Leu	5.6 ± 0.24	2.9 ± 0.22	7.1 ± 0.71	5.6 ± 0.64	4.5 ± 0.44	4.0 ± 0.02	6.4 ± 0.60	4.9 ± 0.02
Lys	5.4 ± 0.25	3.3 ± 0.22	6.9 ± 0.57	6.1 ± 0.65	4.5 ± 0.35	4.0 ± 0.05	6.3 ± 0.67	5.3 ± 0.37
Met	1.2 ± 0.02	1.3 ± 0.10	1.3 ± 0.22	1.3 ± 0.03	1.1 ± 0.02	1.0 ± 0.05	1.1 ± 0.03	1.7 ± 0.14
Phe	4.9 ± 0.23	1.9 ± 0.12	5.5 ± 0.41	7.4 ± 0.91	3.6 ± 0.39	1.3 ± 0.04	5.6 ± 0.20	5.6 ± 0.79
Thr	2.6 ± 0.20	1.9 ± 0.04	3.4 ± 0.30	4.2 ± 0.53	2.1 ± 0.11	2.4 ± 0.03	3.1 ± 0.46	3.7 ± 0.33
Trp	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
Val	3.7 ± 0.16	2.7 ± 0.19	4.6 ± 0.22	3.6 ± 0.60	3.1 ± 0.32	3.0 ± 0.02	4.4 ± 0.38	4.3 ± 0.37
TEAA	36.0	21.1	43.7	41.1	29.7	24.4	41.0	36.1
Ala	3.1 ± 0.16	4.2 ± 0.25	3.9 ± 0.33	6.1 ± 0.63	2.7 ± 0.12	3.4 ± 0.03	3.6 ± 0.42	5.7 ± 0.34
Asp	8.6 ± 0.44	7.0 ± 0.50	10.6 ± 0.60	9.8 ± 0.90	7.0 ± 0.68	6.7 ± 0.02	9.9 ± 1.09	8.6 ± 0.99
Cys	1.9 ± 0.05	1.4 ± 0.05	2.2 ± 0.31	2.4 ± 0.29	1.5 ± 0.14	1.6 ± 0.13	1.5 ± 0.05	1.8 ± 0.13
Glu	12.2 ± 0.66	8.2 ± 0.47	14.3 ± 1.04	11.4 ± 1.02	9.7 ± 1.12	6.5 ± 0.02	14.7 ± 1.53	11.4 ± 0.09
Gly	3.0 ± 0.16	2.6 ± 0.21	3.7 ± 0.27	3.9 ± 0.44	2.5 ± 0.13	2.4 ± 0.01	2.6 ± 0.40	3.5 ± 0.29
Pro	3.1 ± 0.18	2.7 ± 0.24	4.0 ± 0.15	2.8 ± 0.55	2.5 ± 0.27	2.5 ± 0.01	3.8 ± 0.42	3.2 ± 0.24
Ser	3.5 ± 0.15	2.7 ± 0.18	4.3 ± 1.18	4.2 ± 0.27	2.6 ± 0.15	2.5 ± 0.08	4.2 ± 0.48	3.6 ± 0.27
Tyr	2.8 ± 0.19	1.9 ± 0.06	3.4 ± 0.29	2.5 ± 0.30	2.2 ± 0.28	2.4 ± 0.07	3.1 ± 0.26	2.5 ± 0.29
TNEAA	38.2	30.7	46.4	43.1	30.7	28.0	43.4	40.3
TAA	74.2	51.8	90.1	84.2	60.4	52.4	84.4	76.4

Data shown are Mean ± SD; n =10

TEAA (Total essential amino acids), TNEAA (Total none essential amino acids), TAA (Total amino acids)

n.a. – not analyzed

Table 9. Amino acid composition in cotyledons and radicles of some varieties of legumes after germinating 48 hours in g/16g N

Amino acids	*Glycine max*		*Lupinus albus* Amiga		*Pisum sativum* var. *arvense* Arkta		*Faba vulgaris* Piestansky	
	cotyledons	radicles	cotyledons	radicles	cotyledons	radicles	cotyledons	radicles
Arg	7.1 ± 0.63	4.0 ± 0.11	8.3 ± 0.35	6.4 ± 0.69	7.3 ± 0.39	3.2 ± 0.25	8.3 ± 0.22	8.2 ± 0.41
His	1.9 ± 0.13	2.6 ± 0.11	1.5 ± 0.05	1.1 ± 0.10	1.8 ± 0.11	2.2 ± 0.09	2.4 ± 0.09	2.0 ± 0.18
Ile	3.5 ± 0.21	1.9 ± 0.08	3.0 ± 0.06	2.7 ± 0.14	3.5 ± 0.17	2.7 ± 0.18	3.7 ± 0.16	3.4 ± 0.26
Leu	5.9 ± 0.38	2.7 ± 0.14	5.0 ± 0.11	4.2 ± 0.06	5.8 ± 0.24	4.1 ± 0.20	6.3 ± 0.27	5.8 ± 0.32
Lys	4.9 ± 0.32	3.2 ± 0.14	3.1 ± 0.09	3.8 ± 0.07	6.0 ± 0.23	4.5 ± 0.18	5.5 ± 0.20	5.8 ± 0.49
Met	1.5 ± 0.01	1.2 ± 0.04	1.3 ± 0.04	1.3 ± 0.10	1.2 ± 0.04	1.5 ± 0.11	0.9 ± 0.02	1.2 ± 0.07
Phe	4.5 ± 0.30	2.8 ± 0.26	1.5 ± 0.10	3.2 ± 0.07	2.5 ± 0.25	4.3 ± 0.33	3.7 ± 0.26	4.1 ± 0.21
Thr	3.2 ± 0.23	2.5 ± 0.12	2.6 ± 0.08	2.9 ± 0.12	3.0 ± 0.21	3.3 ± 0.09	3.3 ± 0.13	3.3 ± 0.15
Trp	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
Val	3.9 ± 0.24	2.7 ± 0.14	2.9 ± 0.10	2.9 ± 0.26	4.0 ± 0.12	3.8 ± 0.26	4.3 ± 0.11	4.1 ± 0.31
TEAA	36.4	23.6	29.2	28.5	35.1	29.6	38.4	37.9
Ala	3.2 ± 0.17	5.8 ± 0.35	2.3 ± 0.06	3.3 ± 0.21	3.4 ± 0.15	4.1 ± 0.10	3.7 ± 0.09	3.7 ± 0.18
Asp	8.8 ± 0.59	9.1 ± 0.35	6.9 ± 0.13	6.0 ± 0.55	8.9 ± 0.37	7.1 ± 0.21	9.3 ± 0.24	8.2 ± 0.54
Cys	1.9 ± 0.07	1.5 ± 0.12	2.0 ± 0.05	1.7 ± 0.14	2.2 ± 0.03	1.6 ± 0.13	1.6 ± 0.03	1.8 ± 0.10
Glu	16.7 ± 1.00	8.3 ± 0.71	12.7 ± 0.39	8.8 ± 0.47	12.7 ± 0.74	7.9 ± 0.36	13.7 ± 1.07	11.6 ± 1.01
Gly	3.2 ± 0.18	2.2 ± 0.09	2.7 ± 0.06	3.0 ± 0.12	3.4 ± 0.15	2.9 ± 0.14	3.7 ± 0.08	3.5 ± 0.14
Pro	4.2 ± 0.33	2.0 ± 0.19	2.9 ± 0.10	2.7 ± 0.15	3.5 ± 0.30	2.7 ± 0.04	3.9 ± 0.15	3.7 ± 0.21
Ser	3.7 ± 0.28	2.9 ± 0.21	3.3 ± 0.10	2.7 ± 0.20	3.9 ± 0.25	3.1 ± 0.14	3.9 ± 0.14	3.6 ± 0.26
Tyr	3.0 ± 0.17	1.2 ± 0.04	3.3 ± 0.06	2.4 ± 0.16	3.4 ± 0.35	2.5 ± 0.11	3.0 ± 0.11	2.9 ± 0.12
TNEAA	44.7	33.0	36.1	30.6	41.4	31.9	42.8	39.0
TAA	81.1	56.6	65.3	59.1	76.5	61.5	81.2	76.9

Data shown are Mean ± SD; n =10
TEAA (Total essential amino acids), TNEAA (Total none essential amino acids), TAA (Total amino acids)
n.a. – not analyzed

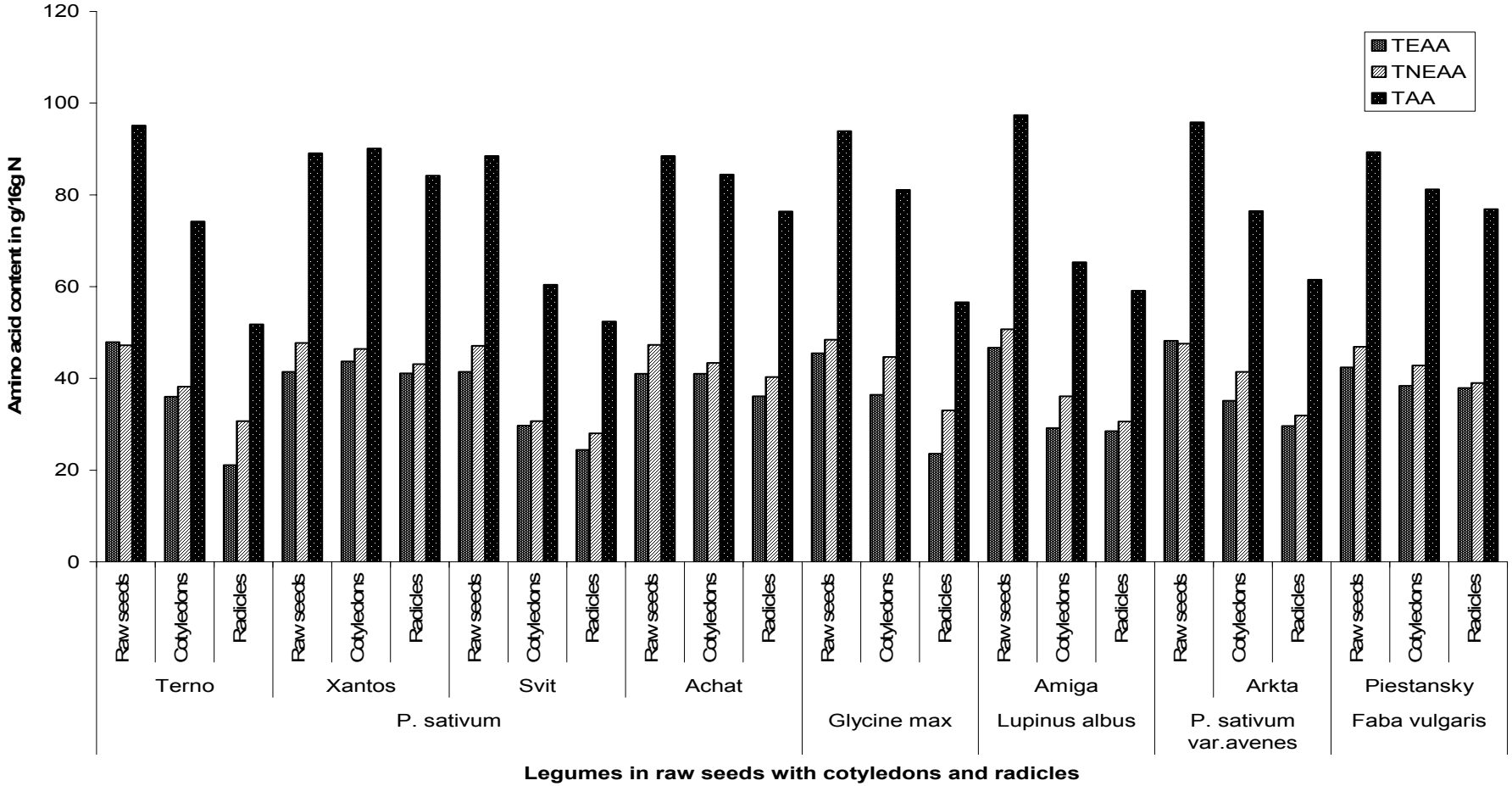

Figure 6. Variation of total essential amino acids, total none essential amino acids and total amino acids of cotyledons and radicles 48 h after germinating with raw seeds of legumes

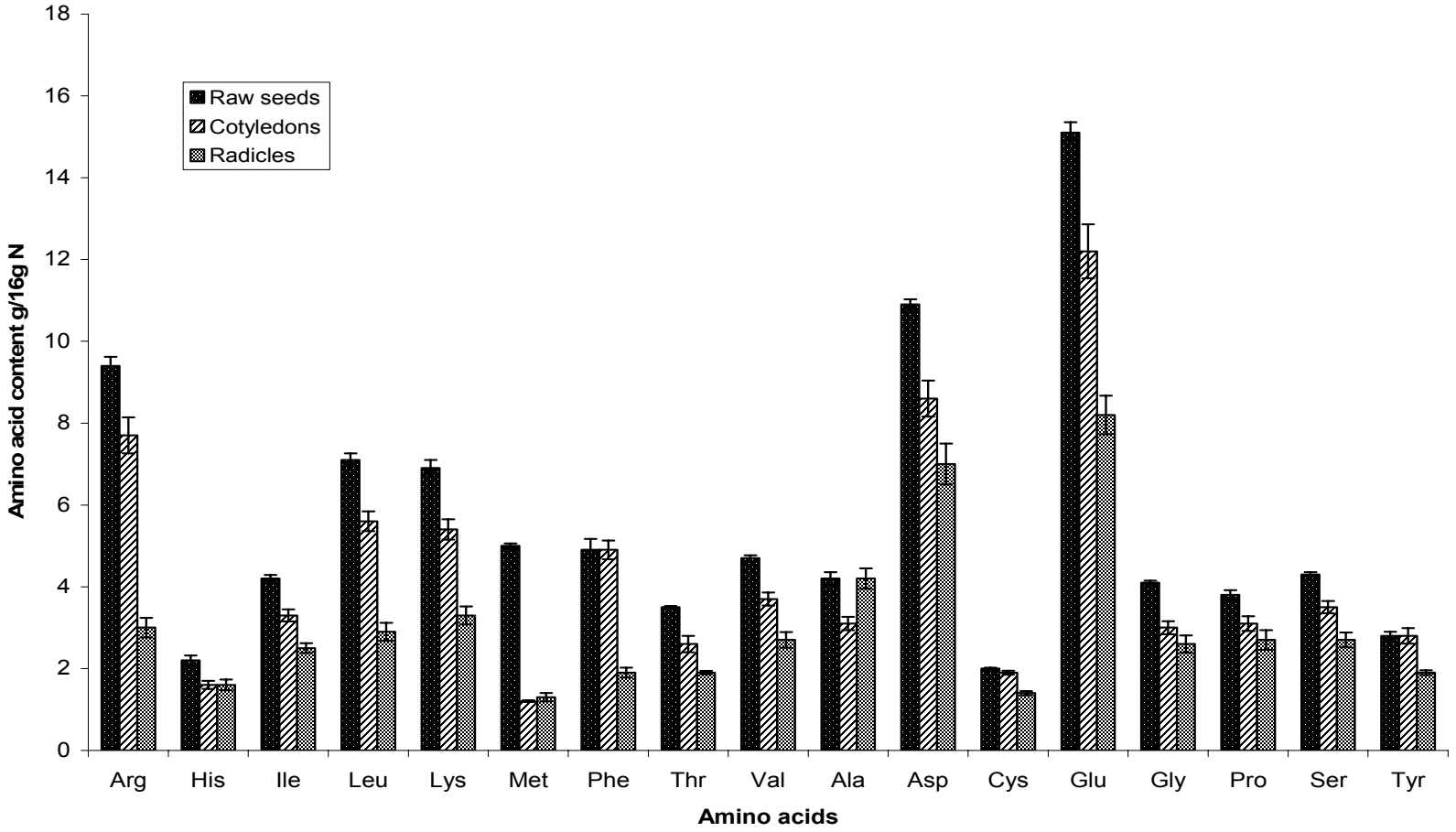

Figure 7. Variation of amino acid content of cotyledons and radicles after germinating 48 h with raw seeds – *P. sativum* (Terno)

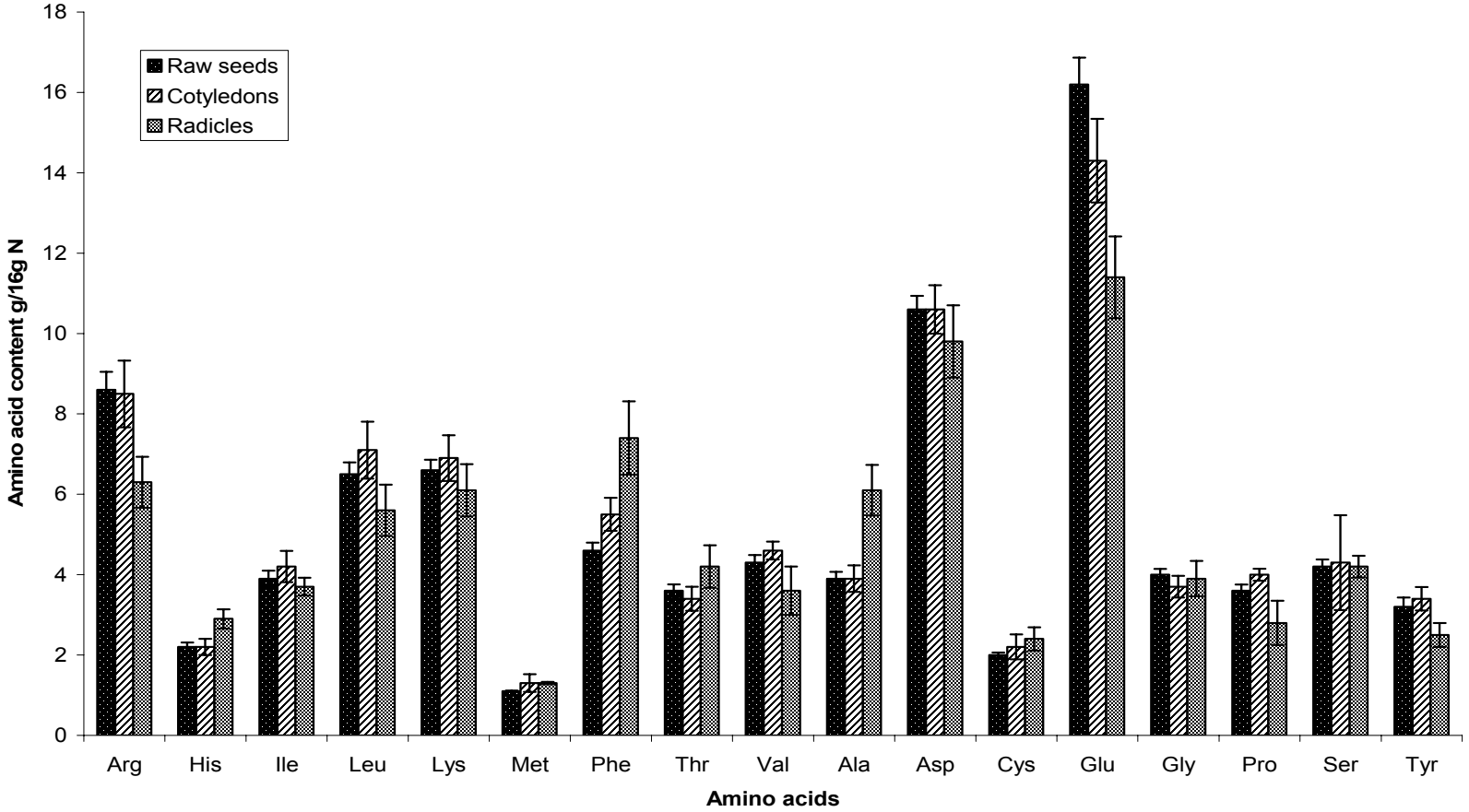

Figure 8. Variation of amino acid content of cotyledons and radicles after germinating 48 h with raw seeds – *P. sativum* (Xantos*)*

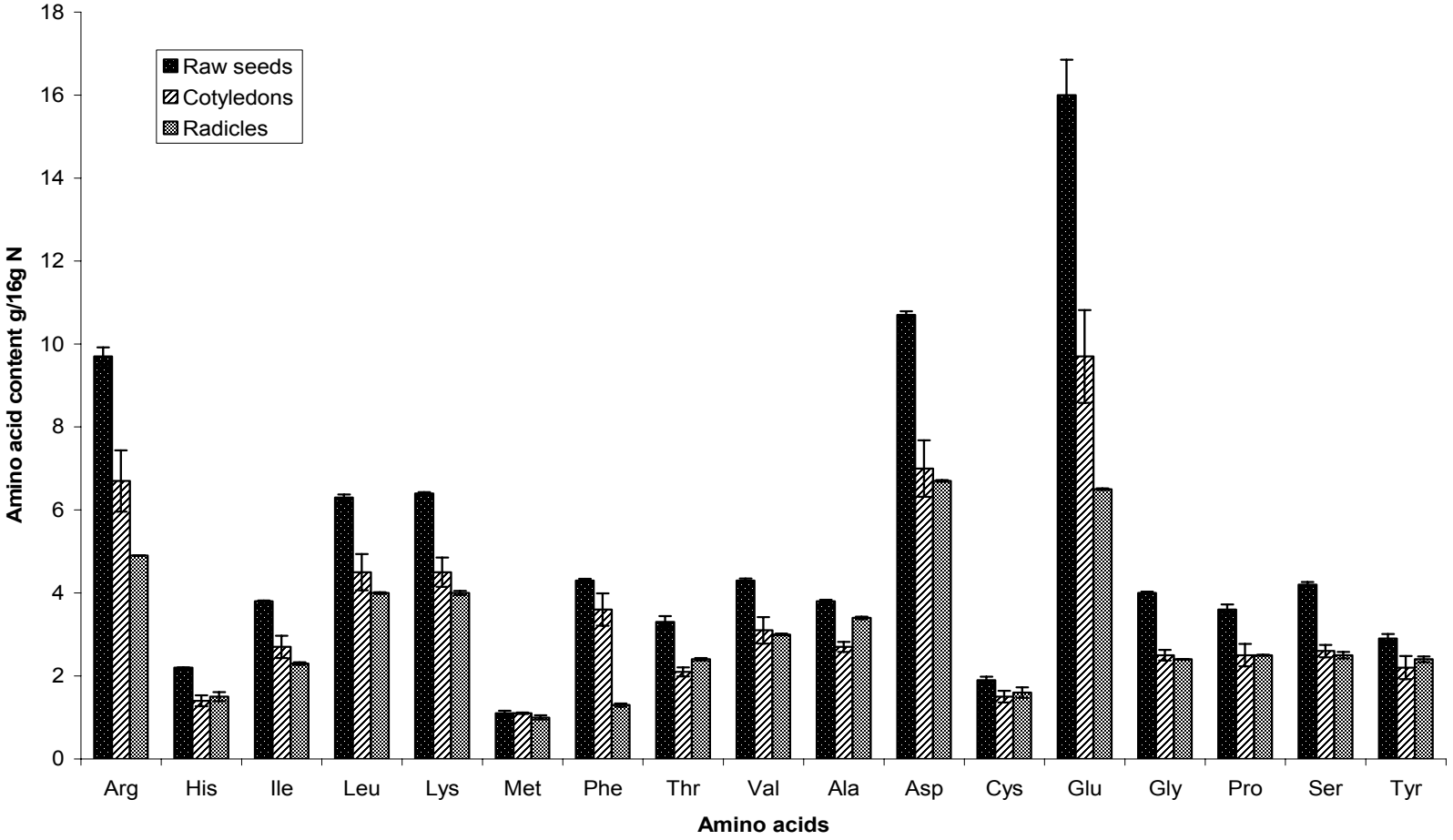

Figure 9. Variation of amino acid content of cotyledons and radicles after germinating 48 h with raw seeds – *P. sativum* (Svit*)*

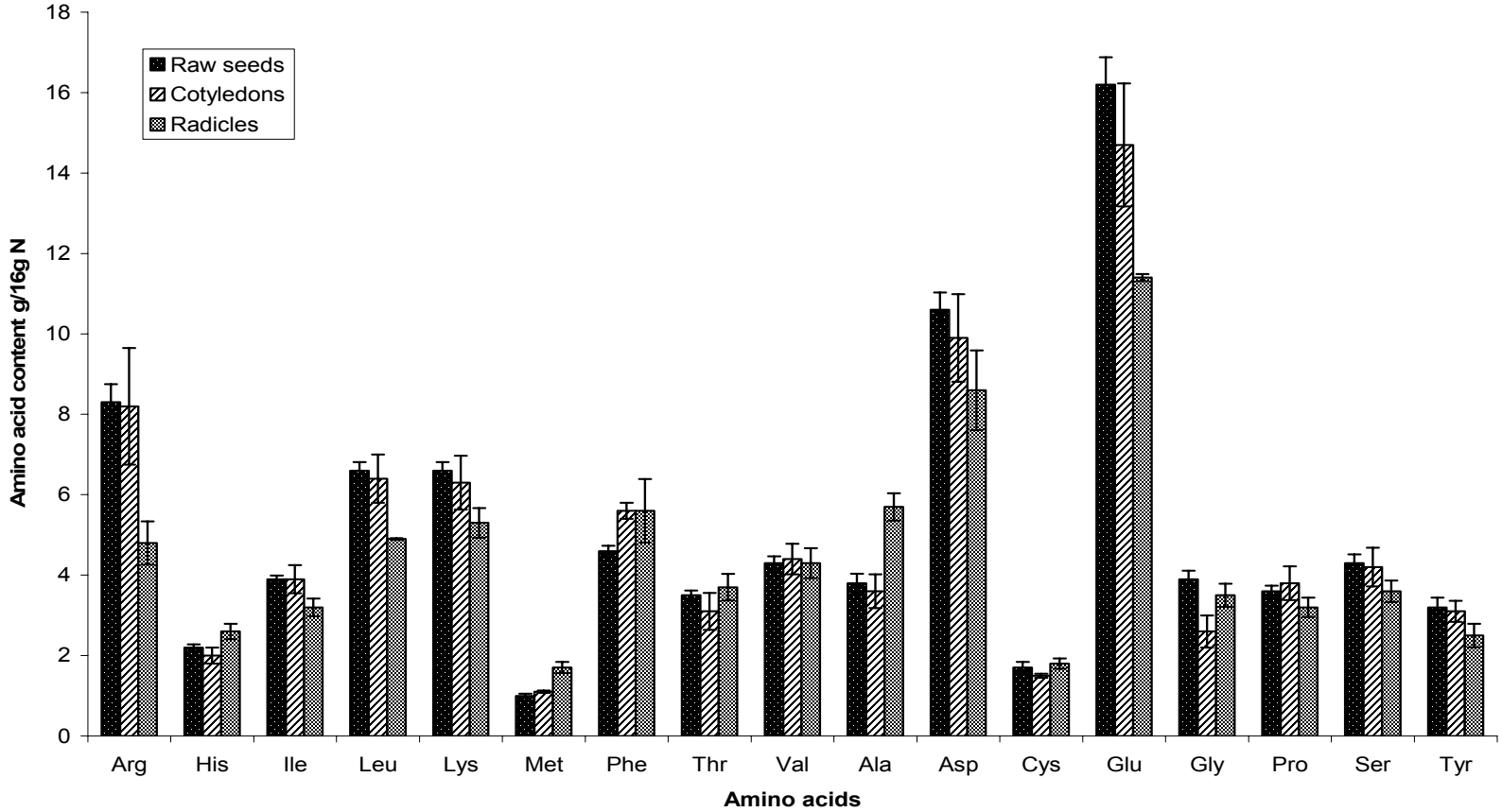

Figure 10. Variation of amino acid content of cotyledons and radicles after germinating 48 h with raw seeds – *P. sativum* (Achat*)*

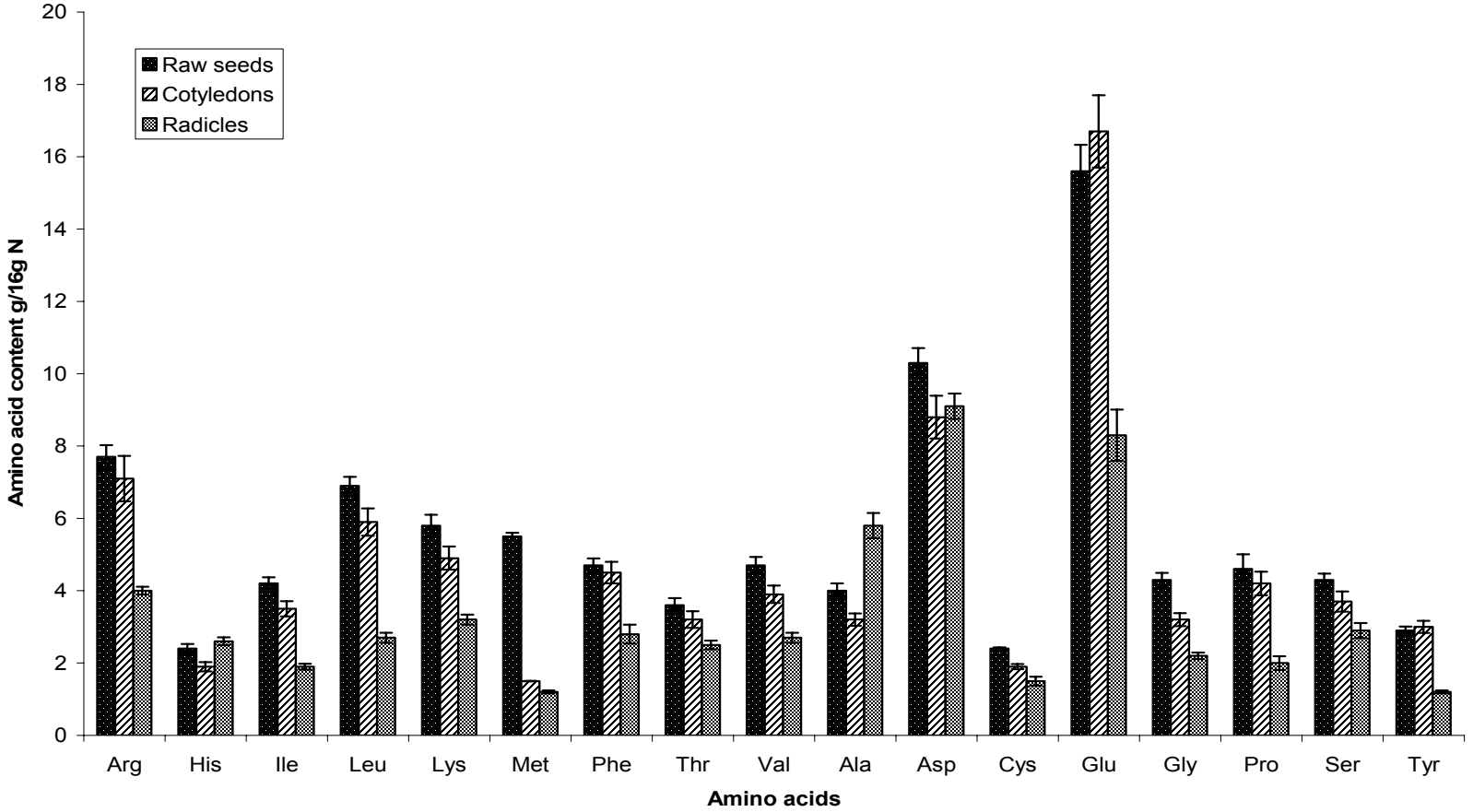

Figure 11. Variation of amino acid content of cotyledons and radicles after germinating 48 h with raw seeds – *G. max*

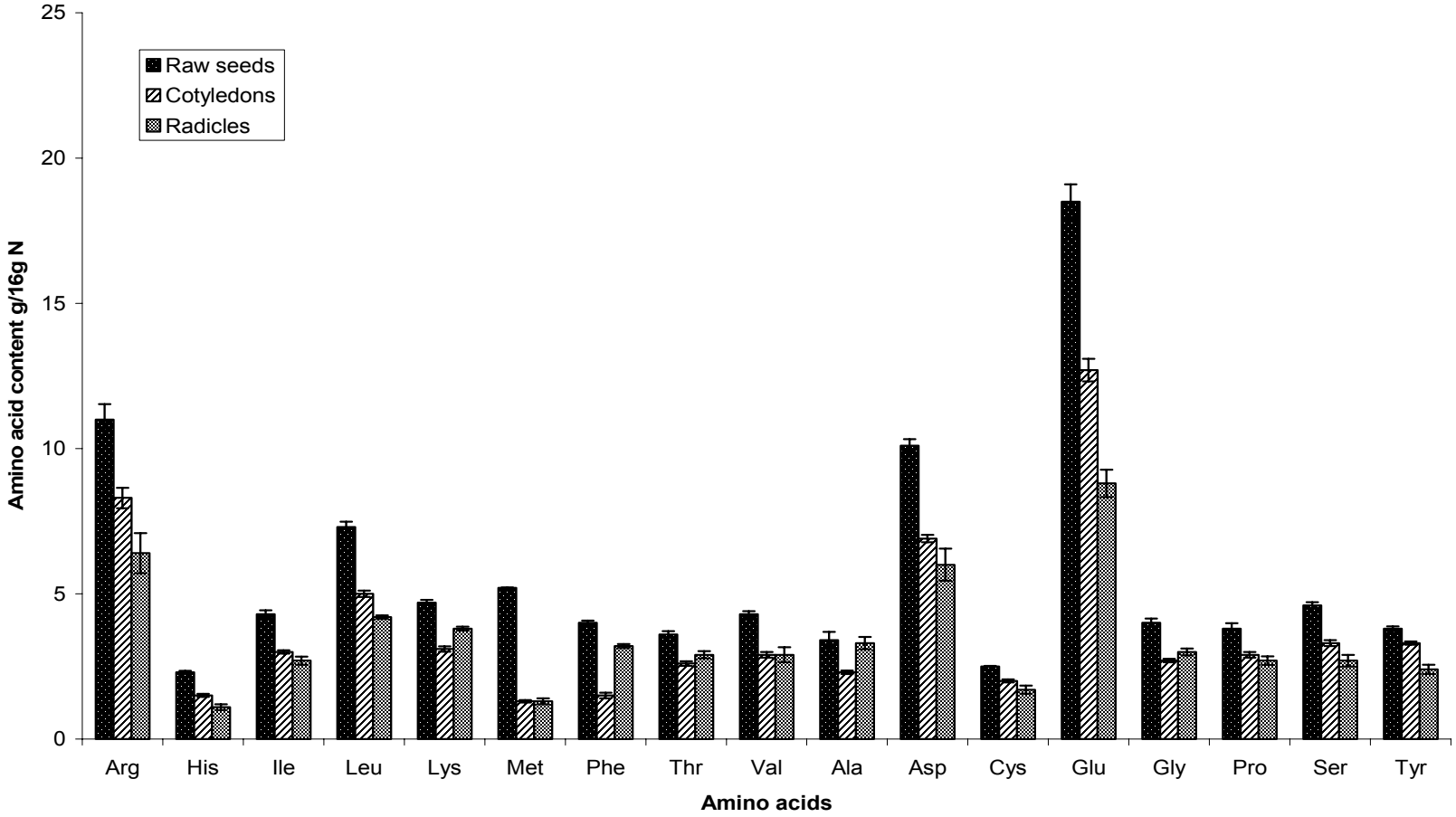

Figure12. Variation of amino acid content of cotyledons and radicles after germinating 48 h with raw seeds – *L. albus* (Amiga*)*

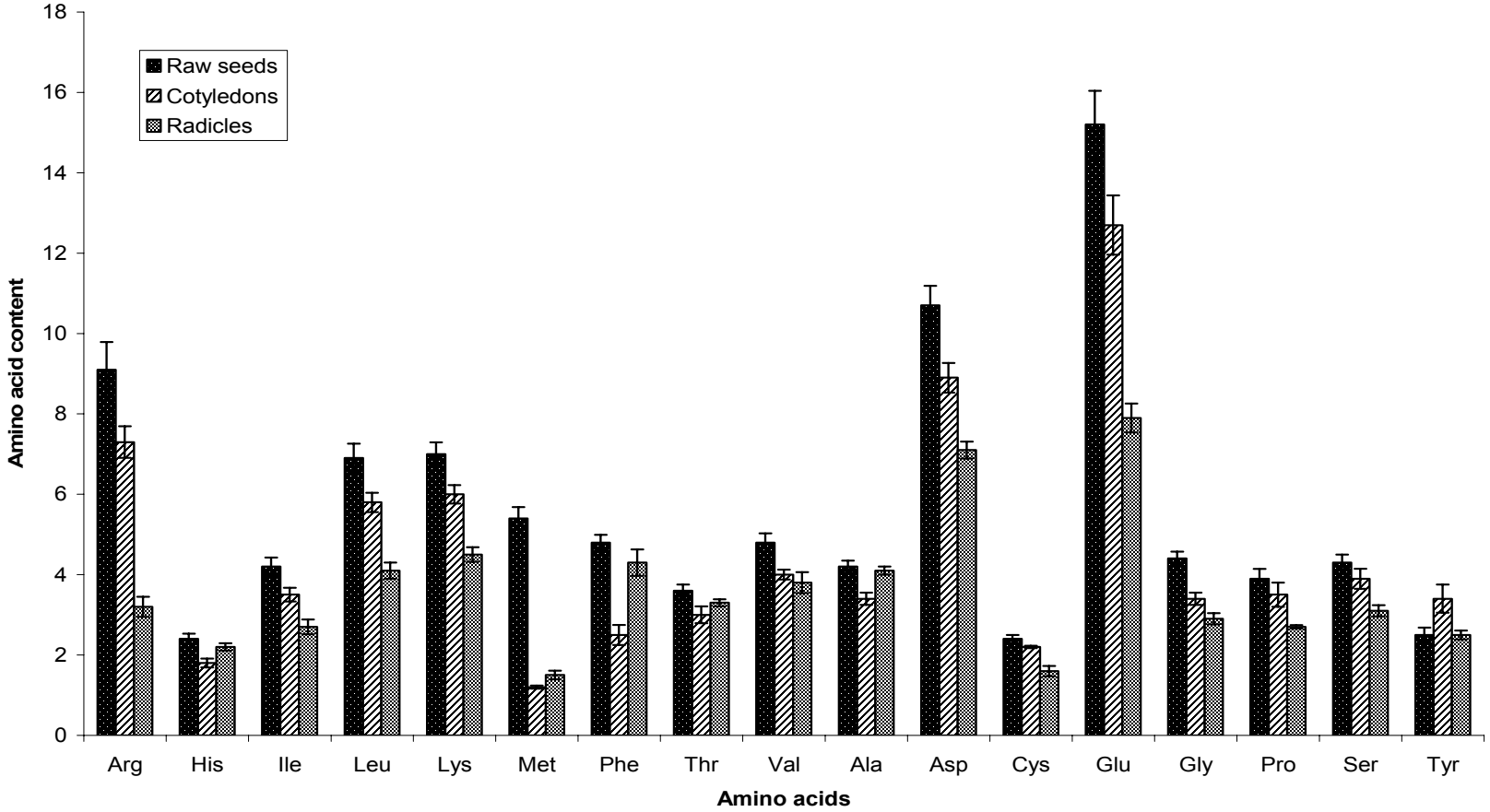

Figure 13. Variation of amino acid content of cotyledons and radicles after germinating 48 h with raw seeds – *P. sativum* var. *arvense* (Arkta)

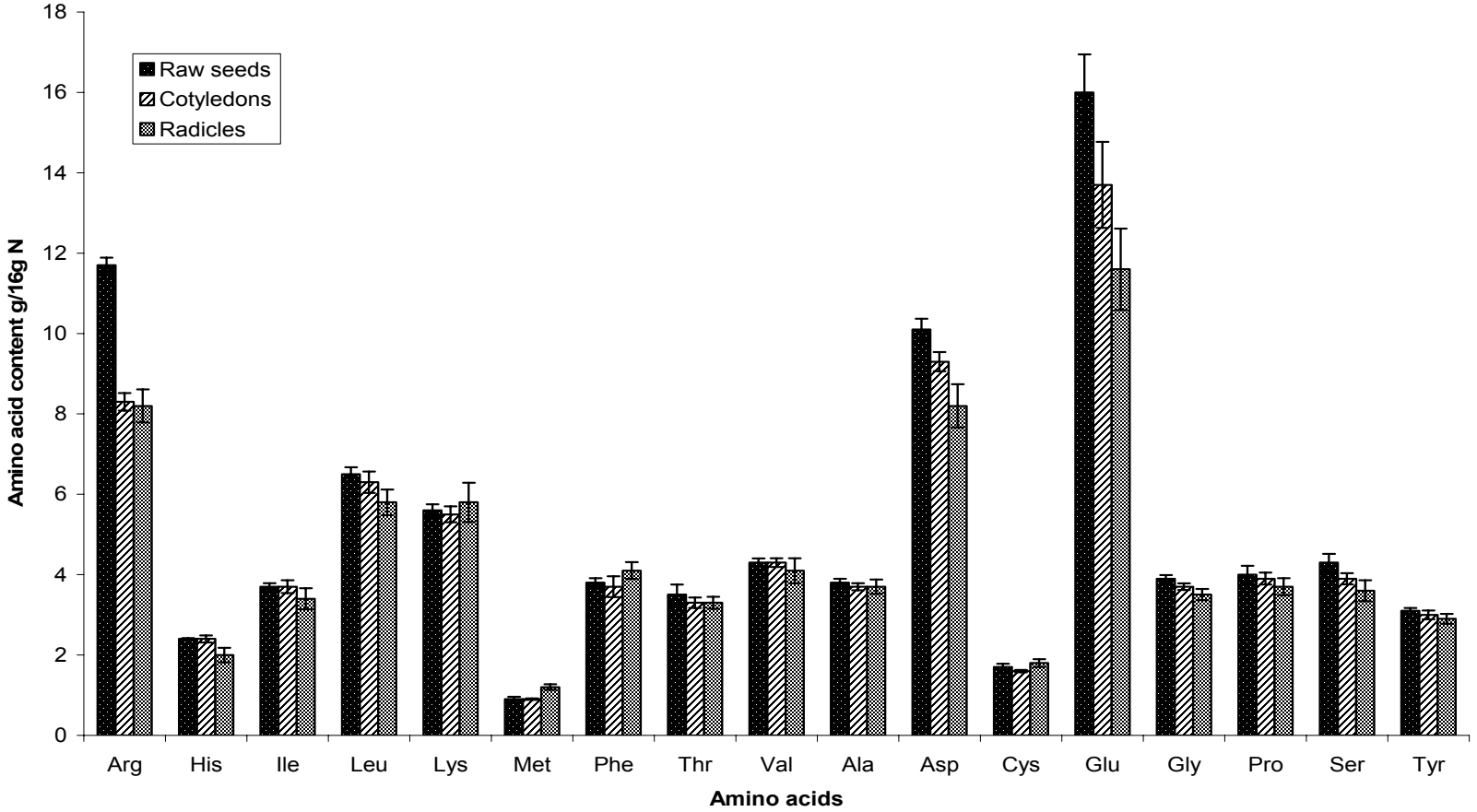

Figure 24. Variation of amino acid content of cotyledons and radicles after germinating 48 h with raw seeds – *F. vulgaris* (Piestansky)

5.2.2 Crude protein of cotyledons and radicles after germinating 48 h

Table 10 describes the concentration of crude protein/100g dry matter found in legumes of both raw seeds and after germination for periods of 2 days for cotyledons and radicles separately.

The results on crude protein of raw seeds of legumes tested were in the range of 21.5 to 34.9% of dry matter are consistent previous reports, those were range from 20-40% [4,74].

As can be seen in the Table 10, the highest % of CP in raw seeds was observed 34.9% in *G. max*, followed by *L. albus, F. vulgaris, P. sativum* (Terno, Svit, Achat, Xantos) and in the *P. sativum* var. *arvense* (Arkta) was the lowest.

As shown in Table 10, CP content was increased in all cotyledons of legumes while it significantly increased in most radicles although decreased the DM content in both cotyledons and radicles. This increase may be related to increased water activity during germination due to hydrolytic enzymes [128]. Similar results were reported in tepary bean in winged bean [125].

After germinating 48 h the CP was increased in most of the cotyledons ranged from 23.1 to 48.0%. According to Trugo et al. [127], the protein of germinated seeds of *G. max* and *L. albus* cultivar Multolupa of Brazil after germinating for 48 h was 41.5 and 34.9% respectively. It was lower in cotyledons of *G. max* (36.3%) and higher in *L. albus* (Amiga) (48.0%) of Central Europe after germinating for 48 h. However, there was a significant increased of CP in radicles of all legumes when compared with both raw seeds and cotyledons except *L. albus* (Amiga*)* and *G. max*. It was highest in the radicles of *F. vulgaris* (Piestansky), 64.9% and the lowest in *G. max*, 32.9%. This increase of CP in radicles may be due to the proteolytic enzyme activities occurs in the cotyledons to breakdown and mobilization of protein in the seeds to develop the sprouts reported for many leguminous species [124]. During germination increase of non protein nitrogen synthesis of new proteins [89,125] mainly up to the third day of germination was observed for Egyptian legume seeds *(Vicia faba, Cicer arietinum* and *Lupinus termes)* [129] may be reasons for the increase CP content of cotyledon and radicles after germinating 48 h. Though a marked loss of proteins and starch from cotyledons was noted as the germination proceeded [86], loss of proteins seemed to be faster than that of starch [130] the results of this study shown an increase of CP in cotyledons after germinating 48 h may be due to increase of availability of protein after soaking and germinating short period. Further, it reveals that germinating short period may be an advantage with a view to retain the protein in cotyledons because it has been observed that germination for 2, 4, or 6 days, with or without light, caused an increase in non-protein nitrogen and a substantial decrease in protein nitrogen due to the hydrolysis of storage proteins that released peptides and free amino acids [87].

5.2.3 *In vitro* protein digestibility and dry matter of cotyledons and radicles

For raw seeds, as shown in Table 10, the lowest IVPD was noted in *P. sativum* var. *arvense* (Arkta) i.e. 54.1% and the highest was noted in *P. sativum* (Svit) (75.0%), which was higher than *G. max* (74.9%). Similarly, based on IVDDM values in Table 10, the lowest IVDDM was noted in *P. sativum* var. *arvense* (Arkta) i.e. 51.1% and the highest was recorded in *G. max* (71.5%).

It is clearly seen that IVPD of cotyledons and radicles increased significantly ($P < 0.05$) after germinating for 48 h in comparison with the results of IVPD values of raw seeds of all the legumes investigated. The IVPD values of radicles of all legumes studied, ranged from 86.7 to 93.4%. The IVPD of cotyledon of legumes ranged from 79.1% to 86.4% and this appreciable increase at early stage of germination is in agreement with raw Indian bean [14]. This improvement in IVPD may be attributed by denaturation, modification extensive break down of protein [1,89] and destruction of the trypsin inhibitor or reduction of tannins and phytic acid [104] during germination. The IVPD values of cotyledons under study was higher when compared with some of the germinated legumes (green gram 72.4%, Bengal gram 73.9%, horse gram 73.8%) [104]. Values for IVPD are vary with different legume species and greatly improve with germination is in agreement with past authors [110] and it was higher when compared with dry matter digestibility in all legumes tested. Low IVPD in raw seeds (54.0-75.0%) in comparison with cotyledons and radicles may be due to more closed structure of polysachcharides in the seeds which may not facilitate to penetrate digestive proteolitic enzymes to digest proteins. Further, the limited susceptibility to hydrolysis by digestive proteases which may be due to structural characteristics of protein, the presence of anti-nutritional seed compounds such as trypsin inhibitors, lectins, polyphenols [91] and the presence of inter-molecular and intra-molecular disulphide bonds [109] may be the reasons for low digestibility of raw seeds. For raw legumes, the IVPD is not dependant on the amount of CP or amino acid content and it may due to the structure of protein which has been reported by many investigators.

The IVDDM values significantly increase in cotyledons ($P < 0.05$) when compared to the respective raw seeds of all legumes studied excepting in *F. vulgaris* which the IVDDM of cotyledon did not significant differ ($P \geq 0.05$) with its respective raw seeds. Further, it has been observed that increase of IVDDM of radicles did not differ significantly ($P \geq 0.05$) with the respective cotyledon of *P. sativum* (Xantos and Svit) and *F. vulgaris.* Similarly the increase of IVDDM of radicles of *P. sativum* (Achat) did not differ significantly with its respective raw seed. Based on the IVDDM values, it indicates that germination may break the closed structure of polysaccharides in the cell wall to facilitate the compounds in the cell to move out.

Table 10. Values and digestibility of dry matter and crude protein of raw seeds, cotyledons and radicles of legumes after germinating 48 hours

Legume	Cultivar		Dry matter (% w/w)			Digestibility of DM (%) *			Crude protein in dry matte (% w/w)			Digestibility of CP (%) *		
Pisum sativum	Terno	raw seeds	90.7	±	0.01	58.3	±	2.51 [a] A	24.2	±	0.51	64.9	±	4.41 [a] A
		cotyledons	41.9	±	0.71	66.2	±	2.22 [b] A	25.5	±	0.54	83.7	±	4.18 [b] A,B
		radicles	12.3	±	1.49	75.3	±	1.88 [c] A	44.7	±	0.61	91.7	±	1.84 [c] A
	Xantos	raw seeds	91.5	±	0.08	60.8	±	1.81 [a] A	21.9	±	0.47	62.6	±	4.40 [a] A
		cotyledons	38.6	±	0.50	70.6	±	2.40 [b] B	23.4	±	0.48	79.1	±	4.56 [b] A
		radicles	12.1	±	0.05	74.9	±	1.11 [b] A	47.4	±	0.52	88.2	±	2.81 [c] A
	Svit	raw seeds	91.3	±	0.09	69.5	±	4.71 [a] B	23.1	±	0.35	75.0	±	3.55 [a] B
		cotyledons	39.6	±	0.49	79.2	±	5.36 [b] C	28.8	±	0.38	82.9	±	0.81 [b] A
		radicles	11.3	±	0.63	74.7	±	1.65 [a,b] A	62.5	±	0.39	91.4	±	1.71 [c] A
	Achat	raw seeds	91.5	±	0.10	69.5	±	3.71 [a] B	22.4	±	0.50	73.7	±	2.21 [a] B
		cotyledons	40.1	±	0.55	79.3	±	4.14 [b] C	23.1	±	0.52	83.1	±	1.39 [b] A
		radicles	10.4	±	0.66	71.1	±	1.37 [a] B	41.6	±	0.57	88.3	±	3.08 [c] A
Glycine max		raw seeds	93.5	±	0.11	71.5	±	2.31 [a] B	34.9	±	0.40	74.9	±	3.95 [a] B
		cotyledons	45.8	±	0.73	76.7	±	2.49 [b] C	36.2	±	0.41	86.4	±	0.48 [b] B
		radicles	20.3	±	1.07	82.9	±	1.98 [c] C	32.9	±	0.46	93.4	±	4.43 [c] A
Lupinus albus	Amiga	raw seeds	92.1	±	0.07	69.6	±	0.47 [a] B	33.9	±	0.52	66.0	±	1.16 [a] A
		cotyledons	38.4	±	2.84	77.6	±	4.36 [b] C	48.0	±	0.60	80.3	±	1.04 [b] B
		radicles	26.8	±	1.05	85.2	±	2.49 [c] C	46.6	±	0.56	86.7	±	0.42 [c] B
Pisum sativum var. *arvense*	Arkta	raw seeds	90.4	±	0.09	51.1	±	2.24 [a] C	21.5	±	0.39	54.1	±	3.84 [a] C
		cotyledons	49.8	±	1.36	56.5	±	2.84 [b] D	19.0	±	0.35	64.5	±	3.13 [b] C
		radicles	13.0	±	0.06	74.0	±	5.08 [c] B	20.2	±	0.40	90.3	±	2.19 [c] A
Faba vulgaris	Piestansky	raw seeds	91.7	±	0.16	67.8	±	3.88 [a] B	29.0	±	0.26	65.6	±	1.86 [a] A
		cotyledons	44.0	±	2.45	70.8	±	3.04 [a,b] B	34.7	±	0.30	82.4	±	4.74 [b] A
		radicles	20.3	±	0.96	76.7	±	4.10 [b] B	64.9	±	0.38	92.6	±	3.90 [c] A

Data shown are mean ± SD; n =10

* Means within a column (in a legume cultivar) with the same superscript letter do not differ significantly ($P \geq 0.05$); means within a column (individually comparison of raw seeds, cotyledons or radicles in 8 legume samples) with the same capital letter do not differ significantly ($P \geq 0.05$)

5.3 Evaluation of nutritional quality of legumes for different cooking methods

5.3.1 Crude protein and dry matter content

According to the results in Table 11, DM of cooked samples after lyophilization was above 95% in all cooking times, irrespective of the method of cooking, for all legumes under study.

Cooking method and time had different effects on the retention of CP content in legume seeds under studied.

The maximum value of CP of *P. sativum* (Xantos), was 25.7% after 30 min of normal cooking, this was the highest increase noted for all times and for all methods of cooking used in this study. Further, CP values of *P. sativum* (Xantos) were higher in lyophilizated samples for all times of normal cooking when compared with all cooking times of pressure cooking and 10-14 min of microwave cooking.

Similarly the highest value of CP for *P. sativum* (Svit) was 25.9% resulting from 10 min pressure cooking and had similar values of CP for all cooking times of all three methods of cooking with few exceptions.

In *G. max* the maximum value of CP attained was 42.0% for 35 min of normal cooking. Slightly lower values of CP were noted in pressure cooking and microwave cooking when compared with normal cooking and there was no significant difference between the CP contents between pressure and microwave-cooked legumes. This is in agreement with similar results for Bengal gram, green gram and horse gram [104]. This may be due to higher cooking time required to inactivate anti nutritional factors in *G. max* to release protein molecules.

The slight decrease of DM% and CP% with the increasing of cooking time may be due to leaching of compounds to the cooking water, which is in agreement with past investigators [6,128]. The slight losses in CP with increasing cooking time in all methods of cooking could be attributed to partial removal of certain amino acids along with other nitrogenous compounds on increasing cooking time, which has been explained by other workers [103]. As the amount of water associated with the protein could be markedly affected the thermal stability of proteins, the temperature and heat-moisture conditions are of great importance [106]. Therefore, losses of nutrients during normal cooking can be controlled by the amount of cooking water [105]. Hence, the combined effect of soaking and minimum amount of water for cooking may result on reduction of nutrient losses.

Table 11. Values (in %) of dry matter content and crude protein content of some legumes with different cooking methods in different cooking times (lyophilizated samples)

Cooking		*P. sativum* (Xantos)		*P. sativum* (Svit)		*Glycine max*	
Method	Time (min)	DM	CP	DM	CP	DM	CP
Normal	20	96.6 ± 1.66	23.8 ± 0.61	97.9 ± 0.72	24.5 ± 2.21	95.9 ± 2.01	40.9 ± 1.05
	25	97.5 ± 0.33	24.4 ± 0.35	97.5 ± 0.40	25.1 ± 1.54	97.9 ± 0.33	40.9 ± 0.73
	30	96.8 ± 0.77	25.7 ± 0.50	94.4 ± 4.20	25.3 ± 0.98	97.1 ± 0.24	40.1 ± 0.19
	35	97.0 ± 0.28	24.2 ± 0.71	97.4 ± 0.15	24.7 ± 0.06	97.3 ± 0.12	42.0 ± 0.20
Pressure	8	96.5 ± 0.49	23.6 ± 0.59	98.0 ± 0.17	25.0 ± 0.03	97.3 ± 0.31	39.4 ± 1.96
	10	97.2 ± 0.32	23.6 ± 0.26	98.1 ± 0.75	25.9 ± 0.10	97.6 ± 0.18	39.4 ± 1.15
	12	97.1 ± 0.38	23.8 ± 0.04	96.8 ± 0.62	22.6 ± 0.04	97.2 ± 0.24	40.9 ± 0.66
	14	96.9 ± 0.59	22.7 ± 0.66	98.0 ± 0.04	25.6 ± 0.62	97.4 ± 0.32	39.9 ± 1.49
Microwave	8	96.7 ± 0.92	24.8 ± 0.24	96.5 ± 0.13	25.7 ± 0.02	96.4 ± 0.01	38.6 ± 1.11
	10	97.8 ± 0.90	22.8 ± 0.06	96.7 ± 0.40	25.6 ± 0.67	95.8 ± 1.26	39.8 ± 0.91
	12	96.8 ± 0.03	23.4 ± 0.72	97.7 ± 0.05	22.6 ± 0.04	97.3 ± 0.06	39.9 ± 0.15
	14	97.1 ± 0.11	23.2 ± 2.26	97.5 ± 0.73	25.2 ± 0.59	96.9 ± 0.06	38.4 ± 1.13

DM – Dry matter
CP – Crude protein

Data shown are mean ± SD; n =10

5.3.2 *In vitro* digestibility of dry matter

As shown in Table 12, values of IVDDM were increased for all three legumes under study after cooking for various times in all methods of cooking.

The maximum value of IVDDM of *P. sativum* (Xantos), in comparison with its respective value for raw seeds (60.8% as in Table 3), was 81.7%. This was obtained for 14 min of pressure cooking. However, it did not differ significantly ($P \geq 0.05$) for 12-14 min of pressure cooking, for 10-14 min microwave cooking and for 35 min of normal cooking. Therefore, 12 min of pressure cooking or 10 min of microwave cooking can be used as methods of cooking to reduce time of cooking while achieving a maximum IVDDM in *P. sativum* (Xantos) after soaking in 0.2% $NaHCO_3$.

Similarly the maximum value for IVDDM in *P. sativum* (Svit), of 86.8% was obtained in comparison with its respective value for raw seeds (69.5% as in Table 3) was for 14 min of microwave cooking. It must be noted that IVDDM values were always above 80% for all cooking methods and for all times of cooking. However, the maximum value did not differ significantly ($P \geq 0.05$) for 20-35 min of normal cooking, 10-14 min of pressure cooking and 12-14 min of microwave cooking. Therefore, 20 min of normal cooking, 10 min of pressure cooking or 12 min of microwave cooking times can be used as methods of cooking to reduce time of cooking while achieving a maximum IVDDM of *P. sativum* (Svit) after soaking in 0.2% $NaHCO_3$.

The maximum value for IVDDM of *G. max*, of 87.9% was obtained in comparison with its respective value for raw seeds (71.5% as in Table 3) was for 14 minutes of pressure cooking. It must be noted that values were above 80.7% for all cooking methods and for all cooking times. However, the maximum value did not differ significantly ($P \geq 0.05$) 35 min of normal cooking, 12-14 min of pressure cooking and 8-14 min of microwave cooking. Therefore, 12 min of pressure cooking or 8 min of microwave cooking can be used as methods of cooking to reduce cooking time while achieving a maximum IVDDM after soaking in 0.2% $NaHCO_3$. The results of IVDDM above are in agreement with the studies of other workers [98,103].

Table 12. Values of *in vitro* digestibility of dry matter (in %) with different cooking method and in different cooking times (lyophilizated samples)

Method	Cooking Time (min)	*P. sativum* (Xantos)	*P. sativum* (Svit)	*Glycine max*
Normal	20	72.3 ± 0.82 [a] A	84.5 ± 2.29 [a,c] B	82.7 ± 0.56 [a] B
	25	72.6 ± 0.46 [a] A	84.5 ± 1.32 [a,c] B	82.6 ± 0.27 [a,b] B
	30	75.2 ± 0.48 [b] A	86.3 ± 0.64 [a] B	84.7 ± 0.40 [b] B
	35	80.7 ± 1.04 [c] A	86.7 ± 0.99 [a] B	87.5 ± 1.10 [c] B
Pressure	8	73.5 ± 1.18 [a] A	80.5 ± 3.77 [b,c] B	81.6 ± 0.43 [a] B
	10	75.1 ± 3.17 [a,b] A	85.8 ± 2.87 [a] B	83.4 ± 1.06 [a,b] B
	12	80.7 ± 0.38 [c] A	85.1 ± 2.14 [a] B	85.1 ± 1.56 [b,c] B
	14	81.7 ± 1.21 [c] A	86.4 ± 0.23 [a] B	87.9 ± 2.65 [c] B
Microwave	8	72.7 ± 0.93 [a] A	80.1 ± 1.39 [c] B	84.7 ± 1.10 [b,c] C
	10	78.7 ± 0.92 [b,c] A	80.7 ± 1.45 [c,d] B	85.6 ± 1.26 [b,c] C
	12	80.5 ± 2.68 [c] A	83.3 ± 1.37 [a,d] B	86.3 ± 1.46 [b,c] C
	14	80.2 ± 4.27 [b,c] A	86.8 ± 1.33 [a] B	84.6 ± 2.17 [b,c] B

Data shown are mean ± SD; n =10

* Means within a column (in a legume cultivar) with the same superscript letter do not differ significantly ($P \geq 0.05$); means within a raw with the same capital letter do not differ significantly ($P \geq 0.05$)

5.3.3 *In vitro* digestibility of protein

As shown in Table 13, values of IVPD were increased for all three legumes under study after cooking for various times in all methods of cooking. This is in agreement with findings of past investigators and it has been reported that cooking significantly improved the protein digestibility (9.9-11.8%) in chick pea [105].

The IVPD values of *P. sativum* (Xantos), in comparison with its respective value for raw seeds (62.6% as in Table 10), was increased up to a maximum of 85.8% after cooking 14 min of pressure cooking. This value is significant ($P < 0.05$). However, for 35 min of normal cooking, or for 12 min of pressure cooking or for 10-14 min of microwave cooking resulted in IVPD values not significantly varying with 84.3% ($P \geq 0.05$). Accordingly, it shows that IVPD in the range of 84.3-85.8% for *P. sativum* (Xantos) can be achieved with less time to cook by cooking for 12-14 min of pressure cooking or 10-14 min of microwave cooking after soaking with 0.2% $NaHCO_3$ for 6 h.

The IVDP values of *P. sativum* (Svit), in comparison with its respective value for raw seeds (75.0% as in Table 10), was increased up to a maximum of 90.1% after 35 min of normal cooking. This value is significant ($P < 0.5$). However, it did not differ significantly ($P \geq 0.05$) with 14 min of pressure cooking (89.8%) and 14 min of microwave cooking (87.5%). Further the IVPD values of all cooking times ranging from 8 to 14 min for pressure cooking and microwave cooking did not differ significantly ($P \geq 0.05$). As such, 8 min of pressure cooking or microwave cooking after soaking with 0.2% $NaHCO_3$ for 6 h can be used to cook *P. sativum* (Svit) with a view to reduce cooking time and retaining higher values of IVPD.

The IVDP values of *G. max*, in comparison with its respective value for raw seeds (74.9% as in Table 10), was increased up to 91.8% being the maximum value, after 14 min of microwave cooking. How ever, it was more or less similar and did not differ significantly ($P \geq 0.05$) to the IVPD values of all the cooking times in microwave cooking, 14 min of pressure cooking and 35 min of normal cooking. It must be noted that the value of IVPD for 35 min of normal cooking varied significantly ($P < 0.05$) with cooking times of 20-30 min of normal cooking. Further, the IVPD values of *G. max* (86.5 - 87.3%) did not significantly differ ($P \geq 0.05$) for 25-30 min of normal cooking and 8-12 min of pressure cooking. Therefore, 14 min of pressure cooking and 8 min of microwave cooking after soaking 0.2% $NaHCO_3$ for 6 h can be used to cook *G. max* with a view to reduce cooking time and retaining above 90% of IVPD.

Table 13. Values of *in vitro* protein digestibility (in %) with different cooking method in different cooking times (lyophilizated samples)

Method	Cooking Time (min)	*P. sativum* (Xantos)	*P. sativum* (Svit)	*Glycine max*
Normal	20	73.2 ± 1.12 [a] A	85.7 ± 0.78 [a] B	75.1 ± 0.33 [a] A
	25	74.5 ± 0.03 [a] A	86.7 ± 0.97 [a] B	87.3 ± 4.53 [b] B
	30	77.8 ± 0.06 [b] A	87.7 ± 0.54 [a] B	86.5 ± 0.43 [b] B
	35	84.3 ± 0.03 [c] A	90.1 ± 0.63 [b] B	91.1 ± 0.63 [c] B
Pressure	8	73.0 ± 0.14 [a] A	84.9 ± 0.28 [a] B	87.1 ± 0.45 [b] C
	10	76.1 ± 1.49 [b] A	87.8 ± 0.44 [a] B	87.5 ± 0.52 [b] B
	12	84.0 ± 0.65 [c] A	87.9 ± 1.75 [a] B	87.5 ± 0.48 [b] B
	14	85.8 ± 0.10 [d] A	89.8 ± 0.58 [a,b] B	90.4 ± 0.14 [c] B
Microwave	8	74.2 ± 0.15 [a] A	83.0 ± 1.44 [a] B	90.6 ± 0.14 [c] C
	10	83.2 ± 0.45 [c] A	85.8 ± 0.20 [a] B	90.8 ± 0.53 [c] B
	12	83.9 ± 0.18 [c] A	85.4 ± 1.27 [a] A	90.7 ± 0.68 [c] B
	14	84.3 ± 0.14 [c] A	87.5 ± 0.33 [a,b] B	91.8 ± 0.64 [c] C

Data shown are mean ± SD; n =10

* Means within a column (in a legume cultivar) with the same superscript letter do not differ significantly ($P \geq 0.05$); means within a raw with the same capital letter do not differ significantly ($P \geq 0.05$)

The improvement IVPD values after all methods of cooking are more or less similar to true digestibility of protein reported by Nagra and Bhatty [47]. He has noted that true digestibility (TD) of protein of peas increased significantly on cooking from 74.7 to 79.8%. Further, the author has reviewed that protein true digestibility of autoclaved peas increased from 85 to 88% is in agreement with the results of pressure cooked samples in this study.

The IVPD values in all methods of cooking are significantly higher than the improvement of protein digestibility (9.9-11.8%) of chick pea [105]. Further, the range of IVPD of the studied legumes (76.11- 86.77%) by 10 min pressure cooking is higher than the highest IVPD values obtained by 10 min of autoclave treatment for (black grams, chick peas, lentils, red and white kidney beans (68.0–76.0%) after soaking in distilled water for 4 h [103]. Therefore, the increase of IVPD of legumes investigated in this study i.e. *P. sativum* (Xantos and Svit) and *G. max* may attributed to the increase in permeability of the seed coat caused by the ionic strength of the soaking in $NaHCO_3$ and heat under pressure may further enhance the leaching out of oligosaccharides into the medium by increasing the permeability of the seed coat [13,131].

The formation of disulphide bonds resulting in the folding of protein molecules causes the decrease of susceptibility to digestive enzymes (reduction of IVPD) [132]. However, the reduction in IVPD with increasing cooking time was not observed in all three methods of cooking used in this study. Therefore, the cooking times used in this study are adequate and further increase in cooking times does not arise.

As shown in Table 13, The IVPD increase in cooked peas, in comparison with raw seeds (Table 10), can be explained that the cooking times used in cooking methods may be adequate, not only to complete elimination of trypsin inhibitor, reduction of tannins and phytic acid contents, but also by the effect of heat on the three dimensional structure of pea proteins, and this reason was noted previous author [102].

According to the sensory attributes (taste, colour, aroma and texture) used to determine the cooking time of each cooking methods for three legumes under study, pressure cooked sample had the best sensory quality for texture even at 8 min of cooking in *P. sativum* (Xantos and Svit) when compared with normal and microwave cooking. Further, when considering the texture of three legumes after cooking, 35min of normal cooking was the same as 8-14 min of pressure cooking. It was noted that microwave cooked legume seeds of every variety under study, had not gained the same softness in texture as in pressure cooked samples. This is in agreement with ABD EL- Moniem [133]. This study further confirmed the previous research that salts may have an effect on improve the textural qualities of legumes, and also affect the protein content and this has been noted by past investigators [134].

Based on the results of DM, CP, IVDDM, IVPD of all the cooking treatments, pressure cooking seems to be the most effective in improving IVDDM and IVDP by retaining the maximum levels of DM and CP with highest sensory quality.

The reason for improving the digestibility in pressure cooking can be explained as heat under pressure may enhance the leaching out of oligosaccharides into the medium by increasing the permeability of the seed coat. This is in agreement with the improved protein digestibility in autoclaved faba bean, field bean, horse gram, *Phaseolus calcaratus*, *P. angularis* and *M. pruriens* var. *utilis* [13].

Further, it is clear that cooking can be used for the improvement of protein quality of peas and this is in agreement with Nagra and Bhatty [47].

5.3.4 Evaluation of nutritional quality of cooked legumes in comparison to raw cotyledons

Based on the results of IVPD of raw germinated and cooked legume seeds in Table 10 and Table 13, it was revealed that the IVPD improved significantly ($P < 0.05$) by germination and cooked seeds. This improvement may be attributed to the denaturation of protein or destruction of the trypsin inhibitor or reduction of tannins and phytic acid in germination as well as in cooking. This is in agreement with those reported by past authors [92, 135].

Table 10 shows that the differences in CP contents, between raw seeds and those germinated. Table 11 shows the CP of cooked legumes by using normal pressure and microwave cooking. Based on those, the CP content of *P. sativum* (Xantos) after cooking were more or less similar to the cotyledons after germinating (23.4%) for 48 h. However, CP content of *P. sativum* (Svit) after cooking were lower to the respective cotyledons after 48 h germination (28.8%) it indicates that at initial stage of germination there CP increase.

It is noted that IVPD values of cotyledons of *P. sativum* (Xantos) (79.1%) is more or less similar to the IVPD values observed initial time of normal cooking but it was lower when compared with pressure and microwave cooked samples (The initial time interval i.e. 8-10 min). Similarly it was noted that the IVPD is higher in all the methods and times of cooking in *P. sativum* (Svit) and *G. max* when compared with their respective cotyledons after germinating 48 h.

Pressure cooking and micro wave cooking resulted in the maximum improvement in IVPD in comparison with values on germination.

Heat treatment is particularly important in the preparation of legumes for consumption, from the point of view not only of acceptability but also of improvement on protein digestibility.

Therefore, if cooking is employed after germinating in all these three legumes, there is a possibility to further increase the IVPD of cotyledons.

It has been reported that pressure and microwave-cooking further increased the digestibility of germinated legumes, showing an insignificant difference ($P > 0.05$) between the cooking methods [104].

Accordingly, it is recommended to cook the legumes after germinating not only to enhance the digestibility but also to reduce cooking time as the seeds are partially digested in germination process.

6. CONTRIBUTION TO SCIENCE AND PRACTICE

Malnutrition arising out of deficiencies in protein energy is a problem that affects developing countries. Relatively inexpensive sources of protein energy are a solution. The studies conducted on *Pisum sativum* (Terno, Xantos, Svit, Achat), *Glycine max*, *Lupinus albus* (Amiga), *Pisum sativum* var. *arvense* (Arkta), *Faba vulgaris* (Piestansky), reveal that they are rich sources of protein. Therefore, these legumes in particular and legumes in general can contribute to alleviate the problem of protein energy malnutrition in developing countries.

The following are findings of this study that can be made use of in practice.

- Germination of these legumes for 48 h, which is a simple process, a technique that can be performed without much investment, enhances the nutritive value of raw seeds. This process can be adopted not only on commercial scale but also domestically for day to day consumption.
- Use of 0.2% $NaHCO_3$ as soaking medium instead of pure water alone was found to be effective in reducing soaking time to 6 h and it was adequate to reduce cooking time using normal cooking, pressure cooking and microwave cooking. Use of $NaHCO_3$ of appropriate concentration as a soaking medium is effective for other legumes as well.
- Considering the retention of optimum protein quality, measured by the CP content as well as IVPD results, pressure cooking between 8-12 min, was found to be the most effective method that can be used for *P. sativum* (for both Xantos and Svit) and *G. max* after soaking in 0.2% $NaHCO_3$ for 6 h.
- Noting that in an average household the only available method cooking is normal cooking, recommended times of cooking for *P. sativum* (Xantos) and *G. max* is 35 min and for *P. sativum* (Svit) is 20 min after soaking with 0.2% of $NaHCO_3$ for 6 h.

P. sativum (Peas) is the widely grown legume in Central Europe. Amongst the legumes investigated in this study, *P. sativum* was found to have a very high protein quality. Hence, it can be used as an alternative to soya bean for both animal feed as well as humans in Central Europe. Further, *P. sativum* can be used with cereals and be used in many food applications as is of *G. max* (soya bean). Therefore, *P. sativum* could be considered as a rich source of plant protein as an effective alternative to *G. max*, which has a high demand in Europe.

FAO recommendation for the daily intake of protein for an adult is 0.8 g/kg weight of body. Legumes under study could satisfy the daily requirement and therefore could contribute significantly to alleviate the problem of protein malnutrition in the third world and in developing countries.

7. CONCLUSION

All legumes investigated are rich in nutritional value and it improves with germination and with different methods of cooking after soaking in $NaHCO_3$.

The following results were noted as significant for raw seeds of legumes under study:

- Crude protein ranged from 21.5-34.4% and the respective value-ranges of the albumin, globulin, prolamin, glutelin and residue in the legumes tested were 40.3-48.5%, 38.6-42.0%, 3.5-5.3%, 3.4-6.4% and 2.8-7.2 % of the total extractable protein.
- The crude fat content was in the range between 1.13-1.4% excepting for *G. max* and *L. albus*, *G. max* had the highest content of crude fat was 18.7% followed by *L. albus* having 7.4%.
- The crude fibre content ranged from 5.7-7.6% in all the legumes tested and with exception of *L. albus* had 16.2% being the highest.
- The most of all essential amino acid profiles of raw seed proteins compared favourably with FAO/WHO requirements excepting that there is a reduction of phenylalanine in all varieties. The highest TEAA (47.9g/16gN) was in *P. sativum* (Terno) among the *P. sativum* tested. Among the *P. sativum* cultivars, methionin content was highest in *P. sativum* (Terno) i. e. 5g/16gN. *P. sativum* var. *arvense* has the highest TEAA (48.2g/16gN) and *L. albus* (Amiga) has the highest TAA (97.4g/16gN) among the legumes tested.

The following results were noted as significant for germinated legumes of varieties under study.

After germinating for 48 h, CP increased in varying amounts in all the seeds ranging from 23.0 to 48.0% with the exception of *P. sativum* var. *arvense* resulting in a reduction. i.e. 19.5%.

- A significant increase was observed in CP of radicles of all legumes when compared with both raw seeds and cotyledons. It was highest in the radicles of *F. vulgaris* 64.9% followed by *P. sativum* (Svit, Xantos, Achat), *L. albus, P. sativum* var. *arvense* and lowest being in *G. max* being 32.9%.
- The highest TAA content in cotyledons (90.1g/16gN) and radicles (84.2g/16gN) was noted in *P. sativum* (Xantos).
- The highest increase of phenylalanine and alanin was observed in the radicles of *P. sativum* (Xantos) in comparison with raw seeds and cotyledons. The next level of increase of alanin in radicles was found in *P. sativum* (Achat) and *G. max.*
- After germinating 48 h significant increase of IVPD ranging from 86.7 to 93.4% in radicles of all legumes was observed followed by values above

80% in cotyledons when compared with raw seeds which ranged from 54.1 to 75.0%.

Germination is an inexpensive process, in comparison with others. It modifies markedly the protein quality of raw seeds after germinating 48 h in their cotyledons and radicles when compared with the raw seeds of the respective legumes.

The following results were noted as significant in cooked legumes *P. sativum* (Xantos and Svit) and *G. max* with normal cooking, pressure cooking and microwave cooking after soaking in 0.2% $NaHCO_3$ for 6 h.

- Values of IVDDM and IVPD increased for all three legumes under study after cooking in all cooking times for all methods of cooking.
- Pressure cooking and microwave cooking are recommended after soaking in $NaHCO_3$ to reduce cooking times to 8-14 min for *P. sativum* (Xantos and Svit) and *G. max*.
- Pressure cooking (8-12 min) is the most effective in improving IVDDM & IVDP by retaining the maximum levels DM and CP % after soaking with $NaHCO_3$.

Cooking can be used for the improvement of protein quality of *P. sativum* (Xantos and Svit) due to higher IVPD values after cooking and therefore, these legumes can be used as an alternative to *G. max.*

Suggestions for further studies

Further studies on the effect of nutritional quality of *P. sativum* with other processing methods, such as extrusion cooking, may be useful in commercial applications, with the view of its use as an ingredient to make divers products as in *G. max.*

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9. LIST OF PUBLICATIONS OF THE AUTHOR

1. Amarakoon, R., Bunka, F., Kracmar, S. Evaluation of protein quality in legumes by amino acid analysis. *Proteins-2008*, Tomas Bata University in Zlin, Czech Republic, 20-21 May 2008, p. 9-13. ISBN 978-80-7318-706-4

2. Amarakoon, R., Bunka, F., Kracmar, S. Study of amino acid contents in some selected legumes species. *Vitamins-2008*. Tomas Bata University in Zlin, Czech Republic, 9-11 September 2008, p. 108-109. ISBN 978-80-7318-708-8

3. Amarakoon, R., Bunka, F., Kracmar, S. Importance of legumes in human nutrition. *Vitamins-2008*. Tomas Bata University in Zlin, Czech Republic, 9-11 September 2008, 106-107. ISBN 978-80-7318-708-8

4. Amarakoon, R., Bunka, F., Kracmar, S., Kocar, F., Hoza. I.. Study on protein fractionation and *in vitro* digestibility of some selected legumes. Conference in Nitra, 1st April 2009, Slovak Republic 2009. ISBN

5. Amarakoon, R., Kracmar, S., Hoza, I., Budinsky, P. The effect of cooking on *in vitro* digestibility of selected legumes. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, 2009 (accepted to publication).

6. Amarakoon, R., Bunka, F., Kracmar, S., Kocar, F., Hoza. I. Evaluation of protein quality of cotyledons and radicles of selected legumes, *Food and Agriculture,* 2009 (submitted to journal)

7. Amarakoon, R., Bunka, F., Macku, I, Kracmar, S. Methods to eliminate anti-nutritional factors in legumes. *8th International Conference on Risk Factors of Food chain*. September 17, 2008, Krakow, Poland. *Slovak Journal of Animal Science*, 2008, 41, 4.

8. Amarakoon. R., Illeperuma, C. K., Sarananda, K.H. (1999). Effect of Calcium Carbide on ripening and quality of Velleicolomban and Willard mangoes. *Tropical Agricultural Research*-Post graduate institute of Agriculture, University of Peradeniya, Sri Lanka, 1999, vol. 11, p. 54-60. ISSN 1016-1422.

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11. Amarakoon, R., Sarananda K. H., Illeperuma, D. C. K. Determination of maturity indices of Mangoes in Sri Lanka. *Food safety and control*, University of Agriculture, Nitra. 28-29 March 2007, p. 375-379. ISBN 978-80-8069-861-4.

12. Kramarova, D., Amarakoon. R., Rop, O., Hoza, I. Determination of Ascorbic acid by HPLC-ECD in Wallachia Apples. *Vitamins-2007*, University of Agriculture, Prague, 2007, p. 224-225. ISBN 978-80-7194-937-4

13. Sarananda, K.H., Amarakoon, R. Methods to minimise post harvest rot of 3 local cultivars of mango. *Annual symposium of Department of Agriculture (ASDA)*, Department of Agriculture, Perdeniya. Sri Lanka. 1999, p. 327 – 333.

14. Sarananda, K.H., Amarakoon, R. Methods to minimize post harvest rot of mango. Krushi, Department of Agriculture, Peradeniya. Sri Lanka. 1999, p. 5-6.

10. CURRICULUM VITAE

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EDUCATIONAL QUALIFICATIONS

2006 - up to date following PhD in the area of Food Science & Technology under a scholarship offered from Czech Republic

1999 M. Sc. (Food Sci & Tech:), Post Graduate Institute of Agriculture (PGIA), Sri Lanka.
Directed study- 'Post harvest loss reduction in Mango'

1993 B.Sc (Agric sp), University of Peradeniya, Sri Lanka
Project: Physico-chemical & food quality studies of locally grown tomato varieties with maturity

PROFESSIONAL EXPERIENCE:

2001- 2006 Deputy Director (Processing Development), Coconut Development Authority, Sri Lanka

2001 Executive Quality Control, Lanka Canneries Ltd., Sri Lanka

1999-2001 Quality Assurance Manager, Pan-Am Foods Ltd. (LARICH), Sri Lanka

1995-1999 Research Assistant, Food Research Institute, Peradeniya, Sri Lanka

1994-1995 Demonstrator, Dept. of Food Sci & Tech: Faculty of Agriculture, University of Peradeniya, Sri Lanka

SEMINARS AND MEMBERSHIPS IN SRI LANKA

1999 - The application of Hazard Analysis Critical Control Point (HACCP)

2001 - Implementation of quality & safety assurance system in food industry

2006 - Served as a member of the working group committee to formulate standards for coconut milk in Sri Lanka Standard Institute (SLSI)

2005-2006 Served as a member of the Advisory committee in HACCP system certification scheme in Sri Lanka Standard Institute (SLSI)

COLLEGE ATTENDED FOR PRIMARY & SECONDARY EDUCATION

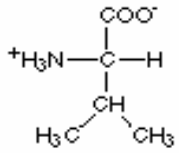
1974-1986 Hillwood College, Kandy in Sri Lanka

11 LIST OF APPENDIX

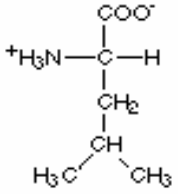
12. APPENDIX

A. Structures of Amino Acids

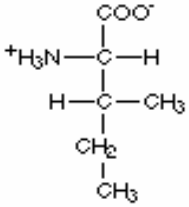
Amino acids with hydrophobic side groups



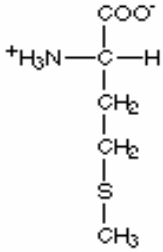
Valine
(val)



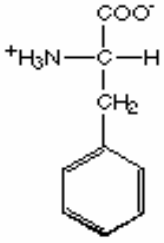
Leucine
(leu)



Isoleucine
(ile)

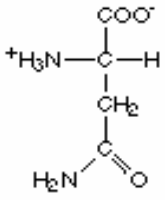


Methionine
(met)

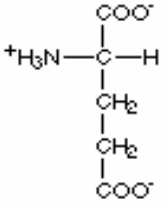


Phenylalanine
(phe)

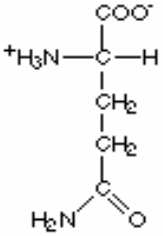
Amino acids with hydrophilic side groups



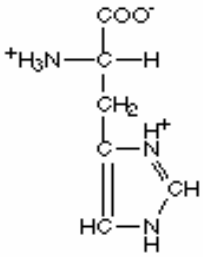
Asparagine
(asn)



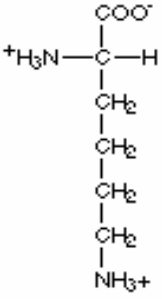
Glutamic acid
(glu)



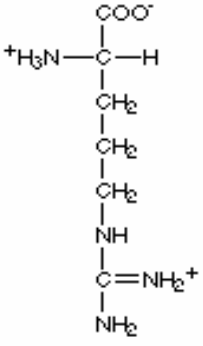
Glutamine
(gln)



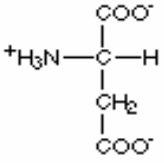
Histidine
(his)



Lysine
(lys)

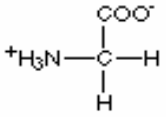


Arginine
(arg)

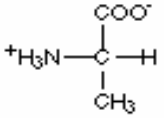


Aspartic acid
(asp)

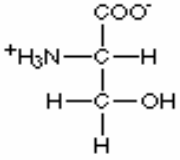
Amino acids that are in between



Glycine
(gly)



Alanine
(ala)



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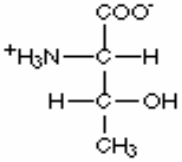
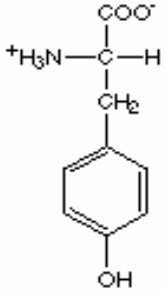
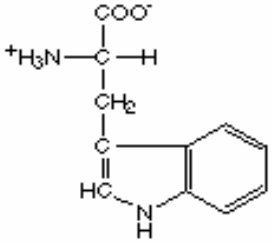
(ser)



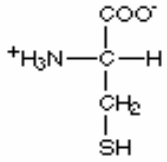
Threonine
(thr)



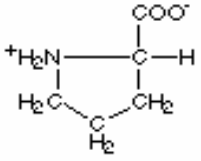
Tyrosine
(tyr)



Tryptophan
(trp)

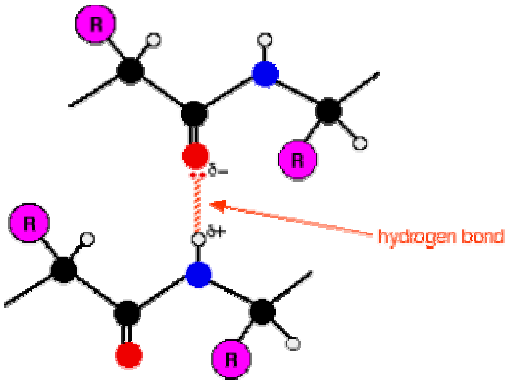


Cysteine
(cys)

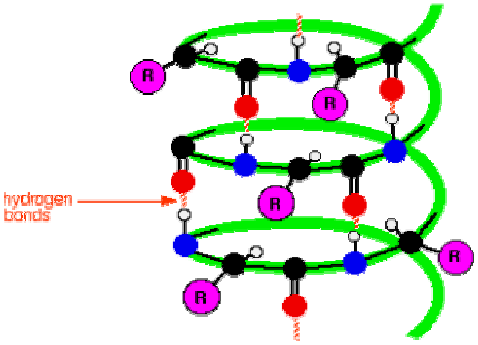


Proline
(pro)

B. **The secondary structure of proteins**

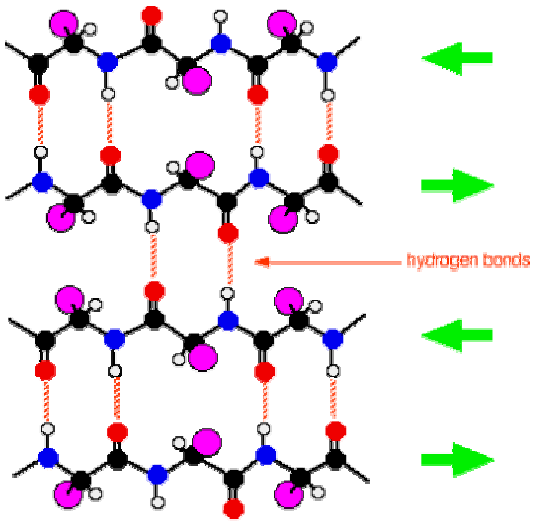


The alpha-helix
In an alpha-helix, the protein chain is coiled like a loosely-coiled spring. The "alpha" means that if look down the length of the spring, the coiling is happening in a clockwise direction as it goes away from you.

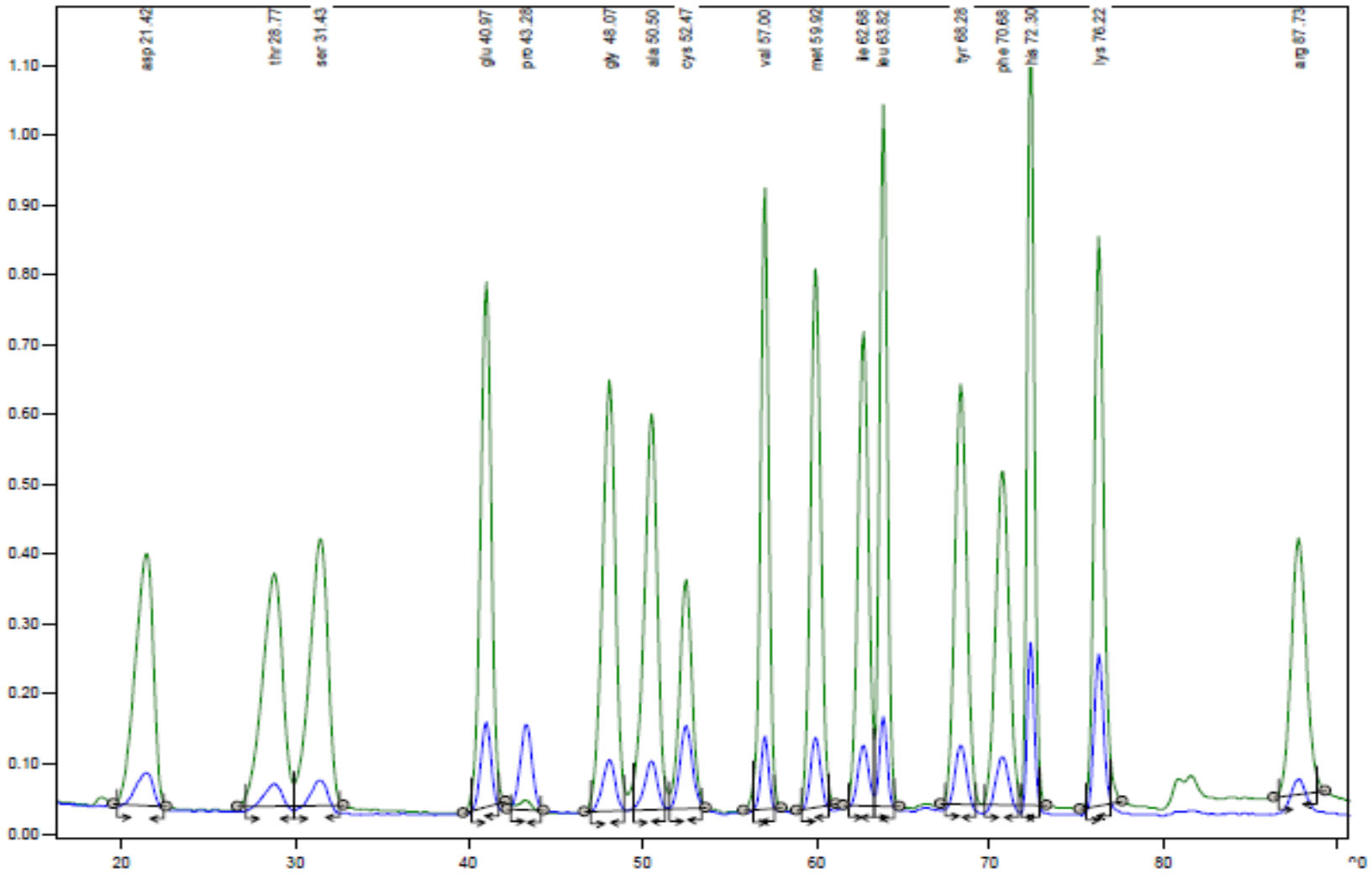


Beta-pleated sheets

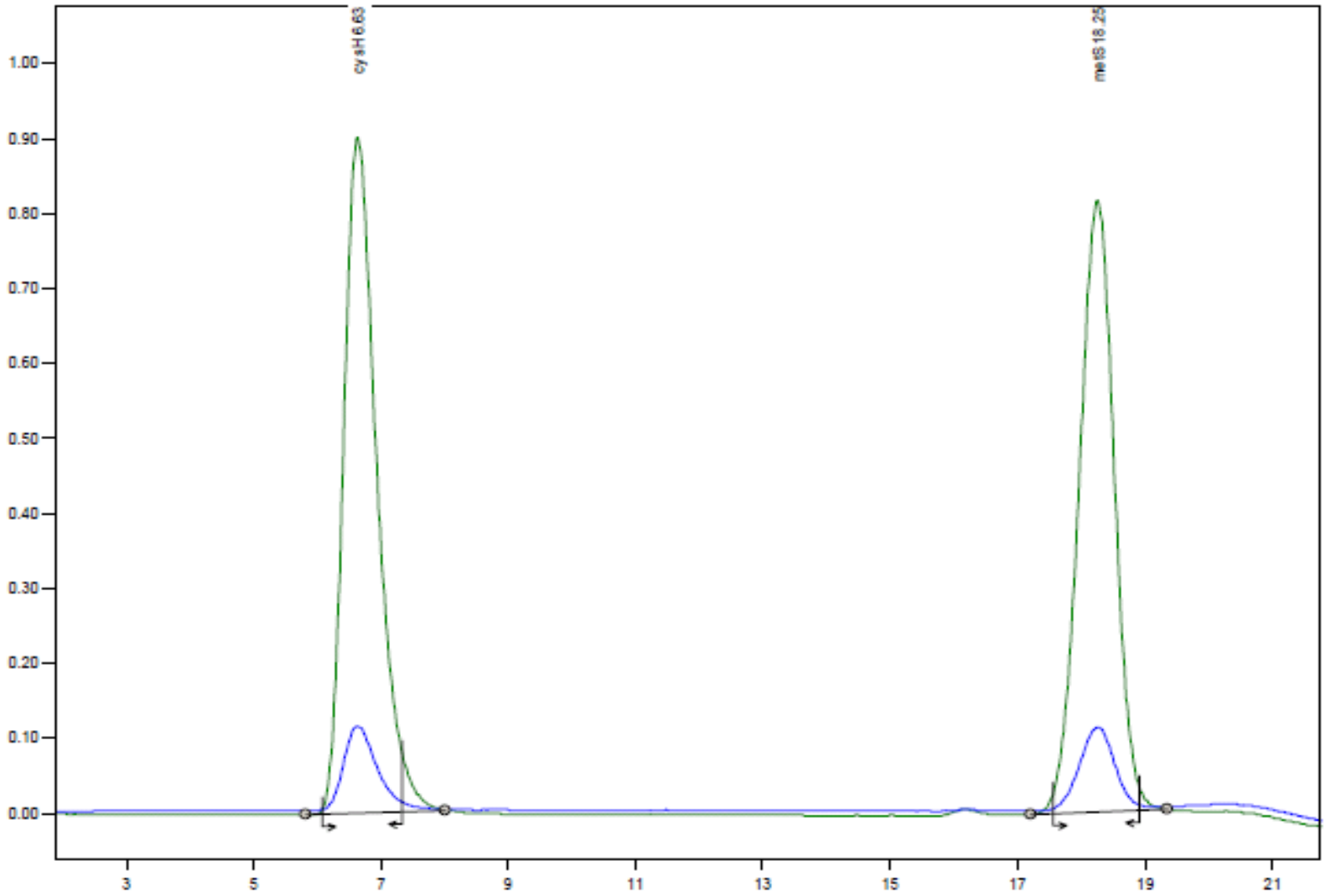
The folded chains are again held together by hydrogen bonds involving exactly the same groups as in the alpha-helix.



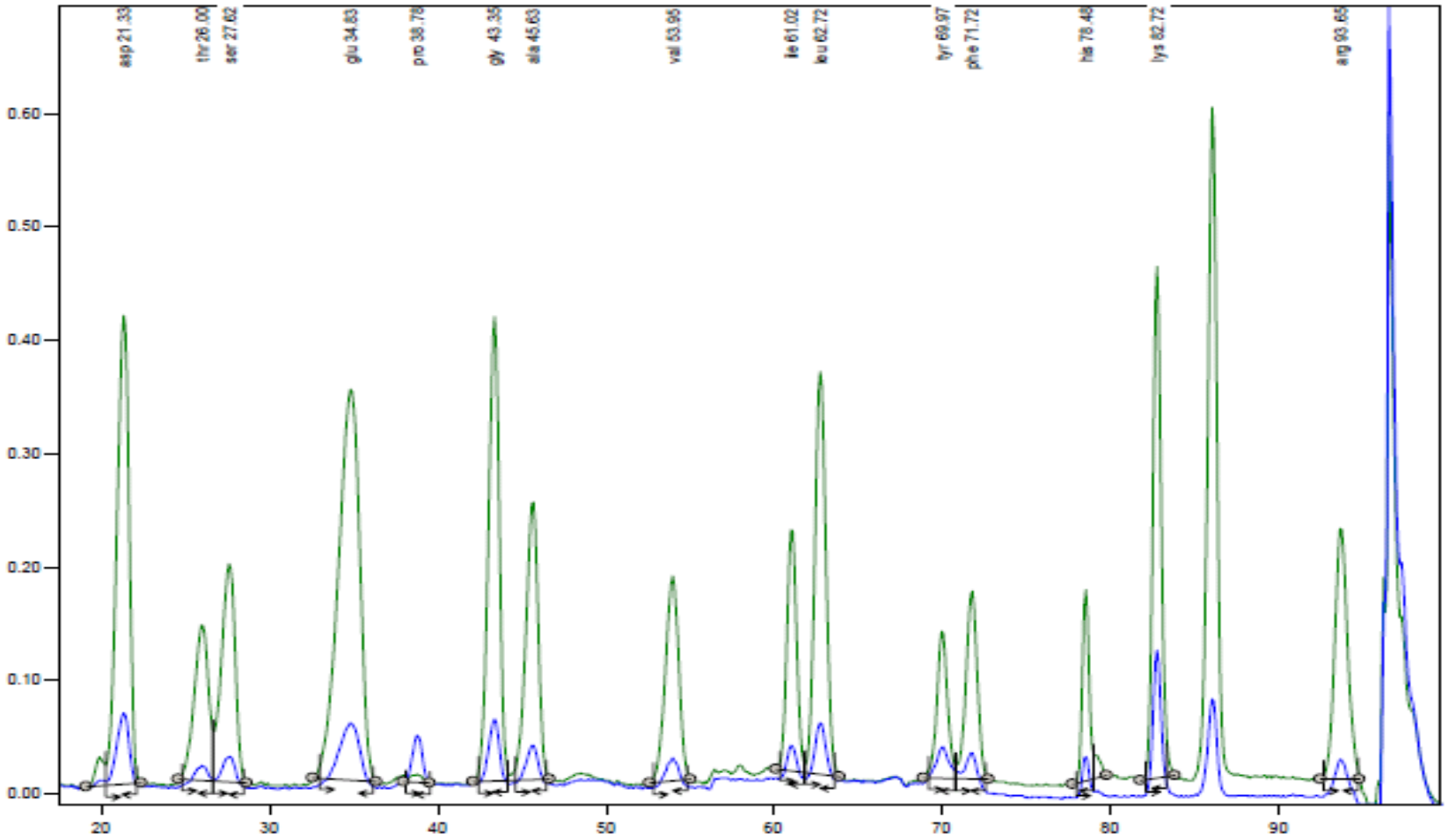
C. Chromatogram of standard amino acids by acid hydrolysis



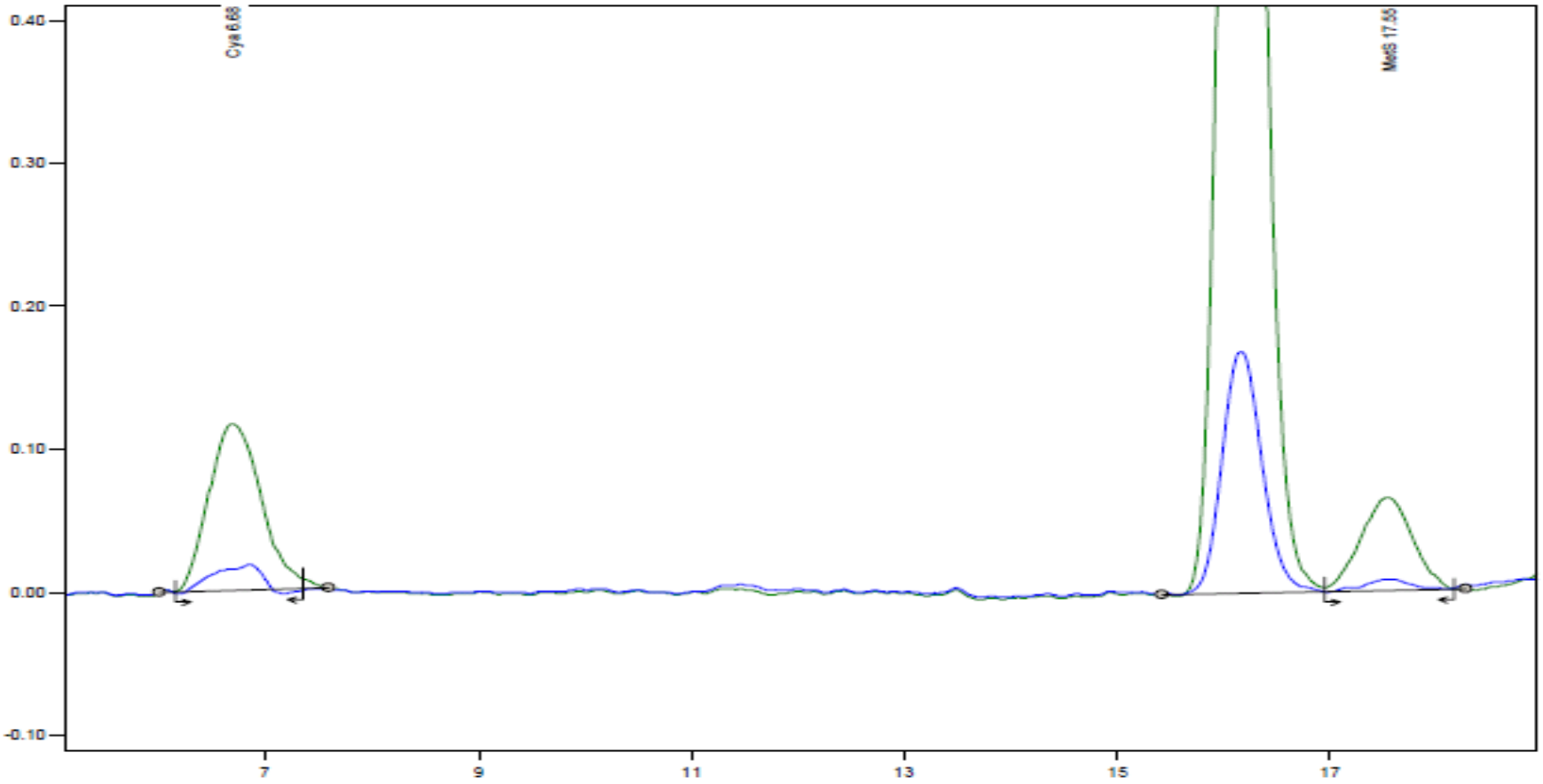
D. Chromatogram of standard amino acids by oxidized hydrolysis



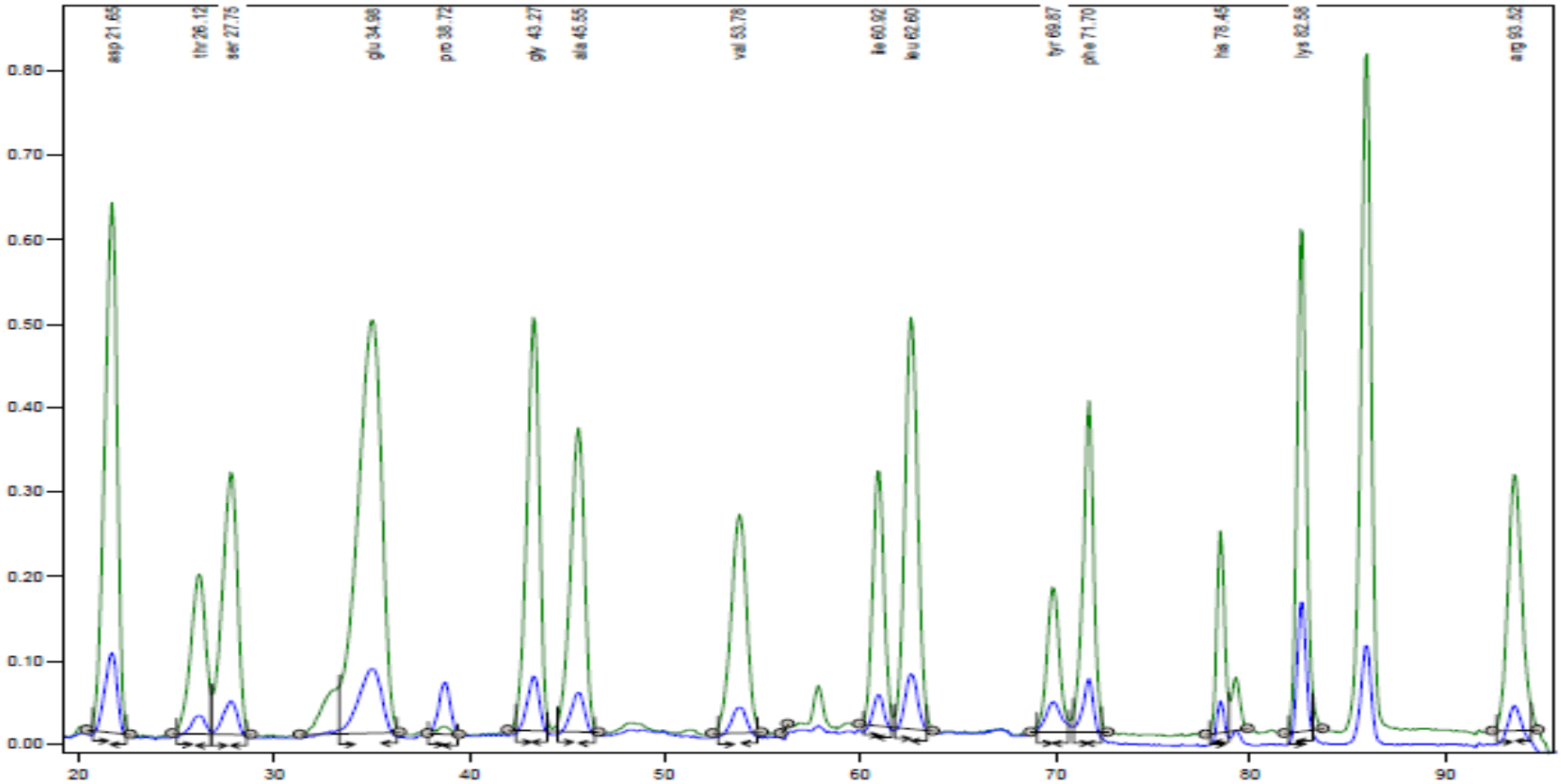
E. Chromatogram of amino acids of raw seeds of *P. Sativum* (Xantos) by acid hydrolysis



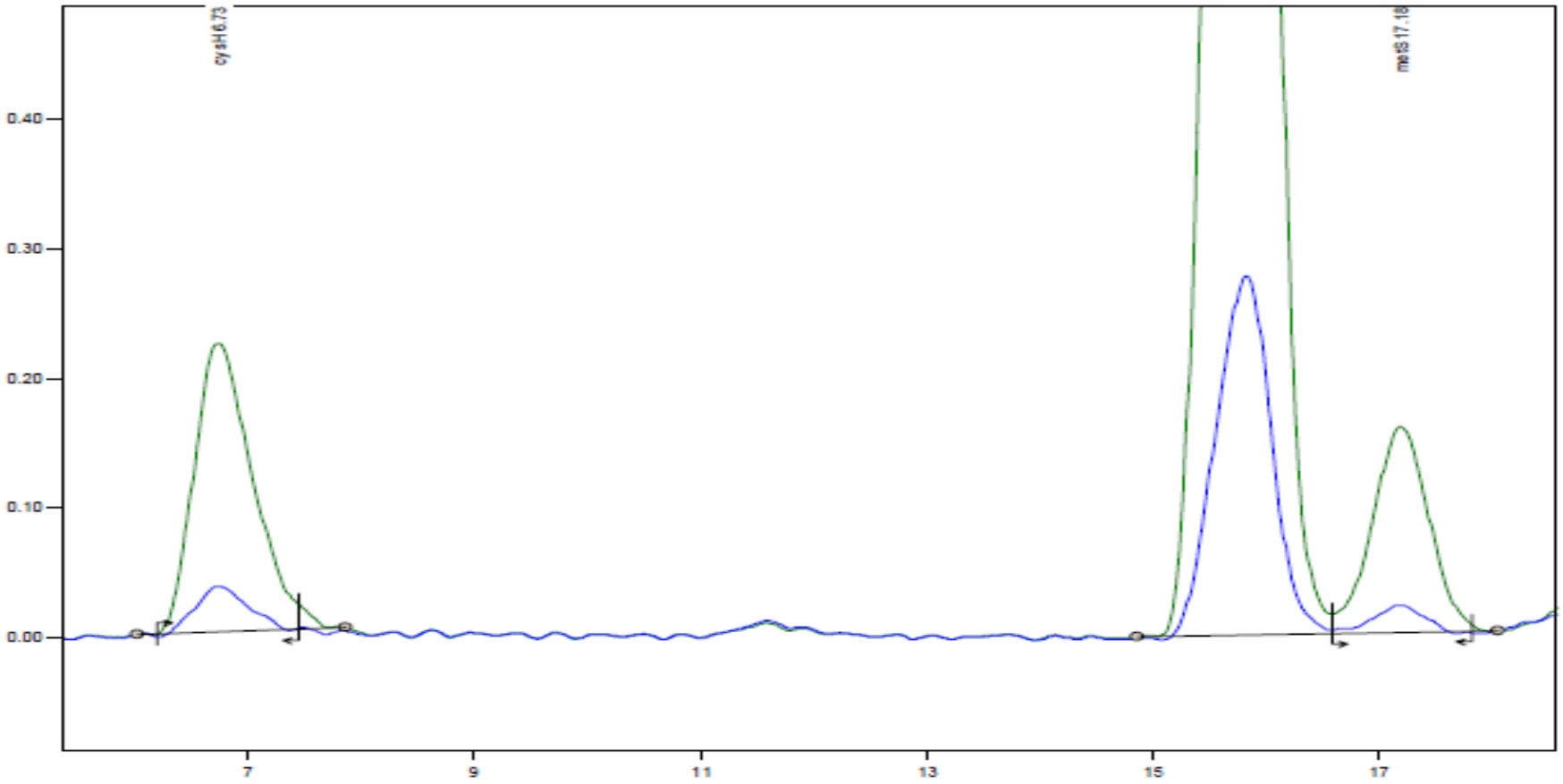
F. Chromatogram of amino acids of raw seeds of *P. sativum* (Xantos) by oxidized hydrolysis



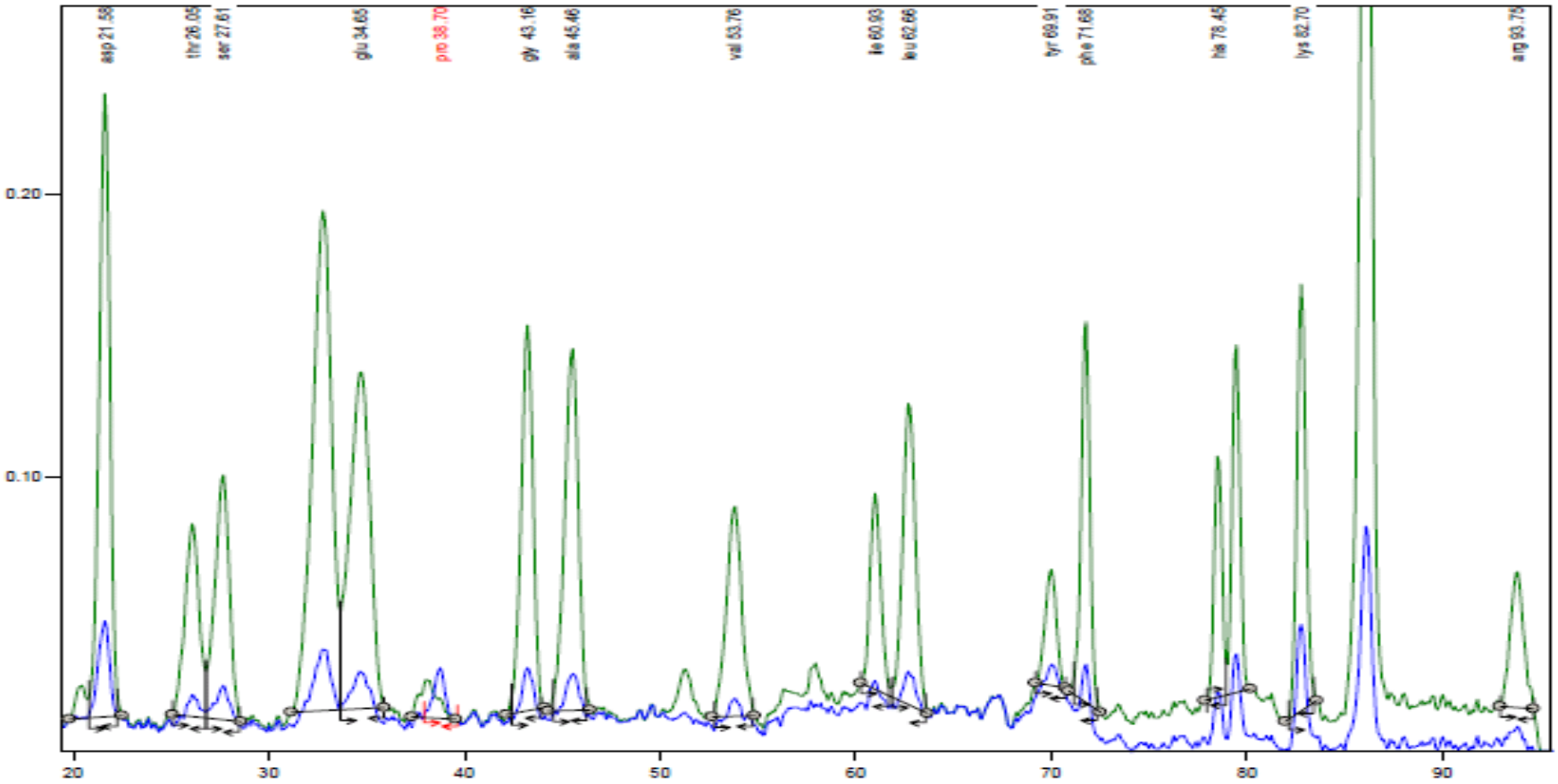
G. Chromatogram of amino acids of cotyledons of *P. sativum* (Xantos) by acid hydrolysis



H. Chromatogram of amino acids in cotyledons of *P. sativum* (Xantos) by oxidized hydrolysis



I. Chromatogram of amino acids in radicles of *P. sativum* (Xantos) by acid hydrolysis



J. Chromatogram of amino acids in radicles of *P. sativum* (Xantos) by oxidized hydrolysis